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UNIVERSITY OF GLASGOW

**THE GROWING AND FEEDING OF LUCERNE, (*MEDICAGO SATIVA*,
SATIVA), TO DAIRY COWS**

By

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Abstract

The purpose of this research was to study the effects of weeds infestation on the growth of lucerne (*Medicago sativa sativa*) in both summer and winter season as well as the effects of lucerne winter harvesting on the growth and yield of lucerne (*Medicago sativa sativa*) in spring. This study further evaluated the effects of ensiling lucerne at dry matter content less than 300 g/kg DM using *Lactobacillus plantarum* MTD-1 as well as the effects of including lucerne (*Medicago sativa sativa*) in dairy cows' total mixed ration, on milk production and enteric methane production. The growing of lucerne to feed dairy cows is expected to ease the current and future feed constraints in dairy farming. The universally increasing demand for milk poses great challenges for the development of cheap, nutritious forage feeds in both the developed and the emerging worlds. The shortage of high quality nutritious forage feeds causes food/feed crises in the emerging world due to the high proportion of cereals used in dairy farming. Therefore, research intensification in the growing and use of both high quality and high yielding forages like lucerne is of paramount importance in dairy farming.

Four experiments were carried out. The first experiment examined the growth rate, effect of weed infestation and winter simulated leaf harvesting of lucerne on early winter growth rate and yield. Lucerne was grown at the SAC Dairy Research Centre, Dumfries, in Scotland, and was harvested at one week intervals in summer 2011 and two week intervals in winter 2012. The second experiment involved the ensiling of lucerne using *Lactobacillus plantarum* MTD-1 microbial inoculant to evaluate its efficacy on nutrient and fermentation characteristics and aerobic stability at dry matter content less than 300 g/kg DM. Lucerne was wilted to about 280 g/kg DM and was inoculated with *Lactobacillus plantarum* MTD-1 at 1×10^6 colony forming units per gram and ensiled for 90 days. The third experiment was a desktop study on the potential effects of replacing maize silage with dried lucerne on milk production and enteric methane production estimated using published equations from the two formulated maize silage and dried lucerne based total mixed rations. The fourth experiment involved the evaluation of the

relationship in enteric methane emission, between the laser methane detector (LMD) and the published equations [Yates *et al.* (2003), Mills *et al.* (2003), Yan *et al.* (2005), Axelsson (1949) and Kriss, (1930)], which was evaluated by measuring and estimating the emissions from the Langhill cows DMI of the total mixed ration (TMR) containing lucerne. The validated LMD enteric methane emissions were used to determine the factors affecting enteric methane emission and measurements of the select and control genetic lines of the Langhill cows.

The study resulted in higher ($P<0.05$) lucerne growth rates and yields in summer and lower growth rate and yield in winter than weeds, and lucerne harvesting at the onset and before midwinter grew taller than the lucerne harvested towards the end of winter. The pH of the *Lactobacillus plantarum* MTD-1 treated silage declined ($P<0.05$) to 5.3 compared to control silages (5.4). The inoculated silages resulted in improved ($P<0.05$) aerobic stability with a mean temperature rise of 4.2°C compared to the control silages (6.7°C) after being exposed to air for eight days.

The inclusion of dried lucerne in dairy cows' diet increased absolute enteric methane by 1.5%, 1.8% and 2.4 % to produce 20, 25 and 30 kg of milk per day but reduced enteric methane by 3.1%, 2.4% and 4.1 % per unit dry matter intake (DMI). The enteric methane production increased by 2.0%, 2.2% and 4.1% per unit milk production as dried lucerne was increasing in the total mixed ration.

The correlation coefficient (r) between the LMD and published prediction equations was high/strong ($P<0.01$) and positive, which ranged from 0.54 to 0.66 except for Axelsson (1949) which had strong and negative correlation ($P<0.01$). The root mean square prediction error (RMSPE), as a percentage of LMD emission, between the LMD and the published equations' methane estimations, as proportion of the observed data, ranged from 2.5% to 34.7%, respectively. The regression slopes of the predicted and observed enteric methane were consistently below the slope of the line of perfect agreement (LPA). The LMD methane measurements showed that the genetic select

Langhill cows emit more ($P<0.05$) enteric methane of 19.9 (9.7) MJ/d than the control cows` 19.2 \pm (SEM= 9.7 MJ/d emission but less ($P<0.01$) emission of 0.5 \pm (SEM=0.07) MJ/d per unit energy corrected milk yield compared to the control cows emission of 0.6 \pm (SEM=0.07) MJ/d. However, the LMD had higher mean emissions measurements than the published equations except for Kriss, (1930).

Table of Contents

Abstract	i
Table of Contents	iv
List of Tables	vi
List of Figures	viii
Acknowledgements	x
Declaration	xi
Glossary of Terms	xii
1. Chapter One Introduction and Literature Review	1
1.1 Introduction	1
1.2 Lucerne, [<i>Medicago sativa sativa</i>]	4
1.3 Silage making in dairy farming	19
1.4 Ensiling of lucerne	31
1.5 The role of forages in dairying	34
1.6 Methane	40
1.7 Research Work	61
2. Chapter Two: Experiment 1. Evaluation of the effect of weed infestation on lucerne yield in summer and winter season and the effect of winter harvesting on lucerne growth rate and yield in spring	63
2.1 Introduction	63
2.2 Materials and Methods	64
2.3 Results	66
2.4 Discussion	71
2.5 Conclusion	73
3. Chapter Three: Experiment 2 Evaluation of the efficacy of <i>Lactobacillus plantarum</i> MTD-1 inoculant/additive on lucerne silage nutrient composition and aerobic stability	74
3.1 Introduction	74
3.2 Materials and Methods	75
3.3 Results	79
3.4 Discussion	83
3.5 Conclusion	89
4. Chapter Four: Experiment 3 A desk top study to evaluate the potential effects of including dried lucerne in dairy cows' total mixed ration (TMR) on enteric methane emission using published equations90	

4.1 Introduction	90
4.2 Materials and Methods	91
4.3 Results	95
4.4 Discussion	100
4.5 Conclusion	104
5. Chapter Five: Experiment 4 Measurement of enteric methane emissions to evaluate the effect of genetic line, dairy cow activity and time of measurement on methane emission of dairy cows offered a high forage total mixed ration (TMR) containing lucerne forage using a laser methane detector	
	106
5.1 Introduction	106
5.2 Materials and methods	108
5.3 Results	111
5.4 Discussion	119
5.5 Conclusions	126
6. Chapter Six : General Discussion	127
7. Chapter Seven: General Conclusions.....	131
Appenidices	157
Appendix 1 Laboratory chemical analyses.....	157
Appendix 2 Diet calculations for Experiment 3.....	158
Appendix 3 Conversion of LMD ppm-m to MJ/ day.....	164

List of Tables

Table 1-1: Area of lucerne grown (in ha) in different countries	6
Table 1-2: Varieties of lucerne available in UK	8
Table 1-3: Summary of the strategies for the management of major pests of lucerne forage	12
Table 1-4: The effect of stage of maturity on lucerne nutrient quality	14
Table 1-5: The nutrient content of lucerne compared to other typical grasses and cereals	14
Table 1-6: The proportion of lucerne harvested at different stages of growth	16
Table 1-7: The effects of different methods of conserving lucerne on quality	18
Table 1-8: Biological processes of ensiling.....	20
Table 1-9: Chemical composition and buffering capacity of lucerne (<i>Medicago sativa sativa</i> , L.) and other typical forage crops in g/kg DM	33
Table 1-10: Recent estimates of natural and anthropogenic universal methane emission, Tg/yr.....	41
Table 2-1: Lucerne and weed yield harvested in winter 2012.....	68
Table 3-1: Lucerne forage and silage pH changes in 250 ml and 5 L silos for day 0, 1, 3 and 7	80
Table 3-2: Lucerne forage and silage pH and nutrient composition.....	80
Table 3-3: Fermentation characteristics of LP MTD-1 Treated and Control silages.....	81
Table 4-1: Feed nutrient composition, cow DMI, feed forage content and milk yield per day for methane emission prediction	93
Table 4-2: Statistical equations used in predicting methane production	93
Table 4-3: Predicted enteric methane emission for best five equations from maize and dried lucerne based feed at 20, 25 and 30 kg milk yield per day	95
Table 5-1: Enteric methane prediction equations	111
Table 5-2: Mean predicted and LMD enteric methane emission in MJ/d ...	112

Table 5-3: Dairy cows DMI (kg/d) and energy corrected milk yield in (kg/d)	112
Table 5-4: The relationship between published equations and LMD (observed) enteric methane emission	113
Table 5-5: The significance of the predicted equations regression slope from the line of perfect agreement (LPA)	114
Table 5-6: Select and Control cows' enteric CH ₄ emission per day, ECM and DMI in MJ/d	118

List of Figures

Figure 1-1: The effect of delayed filling on (A) water soluble carbohydrates and (B) dry matter (DM) loss in maize silage.....	25
Figure 1-2: "Cross linking of lignin to cell- wall polysaccharides is expected to limit cell-wall digestion"	39
Figure 1-3: Microbial fermentation in the rumen.....	43
Figure 1-4: Open Circuit- indirect calorimeter.....	47
Figure 1-5: Ventilated hood or headbox.	48
Figure 1-6: Tracer gas techniques	50
Figure 2-1a: Lucerne and weed yield harvested in 7 weeks of growth in summer 2011.	67
Figure 2-1b: Lucerne and weed DM yields as a proportion of their fresh yield harvested in 7 weeks of growing in summer 2011	67
Figure 2-1c: Lucerne and weed DM yield as a proportion of their fresh yield harvested in 7 weeks of growth in summer 2011.....	68
Figure 2-2: Lucerne and weeds yields harvested at two weeks intervals in winter 2012.	69
Figure 2-3: The growth of lucerne in spring after winter harvesting.....	70
Figure 3-1: Lucerne forage being packed into 5 L bottle silos using a hand presser.....	77
Figure 3-2: The pH changing trend of lucerne silage treated with LP MTD-1 against Control	82
Figure 3-3: Silage aerobic stability / temperature rises at every 12 hour period. 83	
Figure 4-1: Trend of predicted absolute enteric methane emission at 20, 25 and 30 kg milk yield per day	96
Figure 4-2: Trend of predicted enteric methane emission per unit DMI at 20, 25 and 30 kg milk yield	97
Figure 4-3: Trend of predicted enteric methane emission per unit milk yield at 20, 25 and 30 kg milk yield	98
Figure 5-1: The regression and correlation between the LMD CH ₄ measurements and prediction by Yates <i>et al.</i> , (2003) equation.....	115
Figure 5-2: The regression and correlation between the LMD CH ₄ measurements and prediction by Mills <i>et al.</i> , (2003) equation.....	115

Figure 5-3: The regression and correlation between the LMD CH ₄ measurements and prediction by Mills <i>et al.</i> (2003) nonlinear equation	116
Figure 5-4: The regression and correlation between the LMD CH ₄ measurement and prediction by Axelsson (1949) equation.....	116
Figure 5-5: The regression and correlation between the LMD CH ₄ measurements and prediction by Yan <i>et al.</i> , (2005) equation	117
Figure 5-6: The regression and correlation between the LMD CH ₄ measurements and prediction by Kriss (1930) equation	117

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May the Almighty God bless you all.

Declaration

I declare that, except where explicit reference is made to the work of others, that this thesis is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.



Signature:

Printed name: LESTEN ELIEZ CHISOMO CHATEPA

Glossary of Terms

ANOVA	Analyses Of Variance
ADF	Acid detergent fibre
ADL	Acid detergent lignin
C	Control
CH ₄	Methane
CO ₂	Carbon dioxide
C ₆ H ₁₂ O ₆	Carbohydrates/ sugar
H ₂ O	Water
C ₂ H ₄ O ₂	Acetate
C ₃ H ₆ O ₂	Propionate
C ₄ H ₈ O ₂	Butyrate
DM	Dry matter
DMI	Dry matter intake
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organisation
GHGs	Greenhouse gases
NIRS	Near infrared reflectance spectroscopy
ha	hectare
IPCC	Intergovernmental Panel on Climate Change
ILRI	International Livestock Research Institute
kg	kilogram
LAB	Lactic acid bacteria
LCCIEB	London Chambers of Commerce and Industry Examination Board
LMD	Laser methane detector
LP	<i>Lactobacillus plantarum</i>
MBG	Milk Bulking Group
Mcal d ⁻¹	Mega calorie per day
ME	Metabolisable energy
MEI	Metabolisable energy intake
MJ	Mega joule
MT	metric tonnes
MTCO ₂ e	metric tonnes carbon dioxide equivalent
m ²	square metre
m ³	cubic metre
NDF	Neutral detergent fibre
NH ₃ -N	Ammonium Nitrogen
NIAB	National Institute of Agricultural Botany
NPN	Non-protein nitrogen
N ₂ O	Nitrous Oxide
RDP	Ruminal degraded protein
RUP	Ruminal undegraded protein
SAC	Scottish Agricultural College
SEM	Standard error mean
SF ₆	sulphur hexafluoride
T	treatment
Tg	one million metric tonnes

t	tonnes
TMR	Total mixed ration
UNIMA	University of Malawi
UNFCCC	United Nation Framework convention on climate change
VFAs	Volatile Fatty Acids
WSC	Water soluble carbohydrates

1. Chapter One: Introduction and Literature Review

1.1 Introduction

Dairy farming in developing countries faces many growth-limiting factors, such as non-availability of species and breeds of dairy cows, improved dairy breeds, and finance and feed resources (Devendra, 2001). It is reported that universally the non-availability of feeds is a major constraint on animal production (ILRI, 1995). Smallholder dairy production in developing countries is faced with insufficient production of high quality feeds resulting in low milk production (Kumwenda, 1999). Feeding of dairy animals on poor quality feed forages is one of the major constraints in dairy cow production in Africa (Thornton, 2010). The increased production of food of animal origin, such as milk, in response to the growing world population by year 2020, necessitates the use of more feeds than currently (Bradford, 1999). The increased demand for food of animal origin may shift dairy farming systems towards intensified production systems that may move away from grazing systems, to the increasing use of concentrates such as maize to supplement low quality forages (Delgado *et al.*, 1999). It is also important that substantial feed resources are available in the form of high quality forages and other crop residues (Devendra, 2001). Commercial feeds such as dairy mash are too expensive for smallholder dairy farmers in developing countries to buy (Kumwenda, 1999), forcing the farmers to feed the animals with their own food grains for improved milk production.

In the livestock industry system of both ruminant and monogastric livestock in developed countries, including Scotland, cereal grains are used as animal feed (Delgado *et al.*, 1999). The increased demand for milk consumption in developed countries, coupled with the growing demand and production of milk in the developing world, will result in reduction of cereal grains meaning humans will not have enough for food (Brown, 1997; Delgado *et al.*, 1999). This is because it is estimated that animal feed consumption may increase by 292 million metric tonnes by 2020 (Delgado *et al.*, 1999). This will increase the price of maize grain by one-fifth by 2020 (Delgado *et al.*, 1999)

making it harder for smallholder farmers in sub-Saharan African countries like Malawi to feed themselves and their dairy cows.

Although dairy farmers in developed countries seem not to experience constraints in dairy nutrition, the increase in milk production will make cereal grains scarce and prices will increase, making it hard for them to feed their animals by 2020 (Delgado *et al.*, 1999). As the trend of feeding dairy cows on maize grains to produce more milk increases in response to the growing demand, man and animals will universally compete for food (Anderson, 1978).

In normal circumstances, the competition for food grain resources between human beings and ruminant livestock like dairy cows can be alleviated by growing high quality forages such as legumes. Domestic animals, such as dairy cows, use a range of materials which cannot be consumed by human beings as foods, besides feeding on almost more than 33% of the world cereals (Bradford, 1999).

The inclusion of lucerne in dairy cows' total mixed ration (TMR) can reduce the proportion of supplements in the form of cereals like maize, resulting in reduced feed costs (Phipps *et al.*, 1997; Bradford, 1999). The feeding of lucerne to animals results in increased DMI, growth rate and milk yield because of fast rumen fermentation and physical degradation compared to grass forages (Dewhurst *et al.*, 2009). In ruminant nutrition, legume forages such as lucerne have higher feeding value than grass forages because of their rapid particle degradation, faster rumen fermentation and reduced rumen retention time besides having greater voluntary intake (Ulyatt *et al.*, 1973).

However, the high nutrient content of lucerne, in terms of crude protein and minerals, and high digestible energy with low water soluble carbohydrate (WSC) content, gives lucerne a high buffering capacity making it difficult to make good quality silage (Muck and Hintz, 2003).

In the survey study conducted by Phipps *et al.* (1997), cold weather was the main problem associated with lucerne conservation in the UK which resulted in either incomplete wilting or prolonged wilting and loss of leaves. This is because lucerne takes more time to reach the desired dry matter, during wilting, for ensiling in cold weather than in warm weather. In prolonged wilting, more water soluble carbohydrates are depleted through respiration (Phipps *et al.*, 1997). Water soluble carbohydrate in forages is the substrate for both homofermentative and heterofermentative lactic acid bacteria during ensiling, therefore because if depleted during wilting the resulting silage may be spoiled (Kung, 2010). However under warm/hot climatic conditions, the lucerne forage may over- dry during wilting, resulting in the loss of more leaves as it becomes brittle and the leaves shatter easily (Lancefield *et al.*, 2009). The high buffering capacity in lucerne requires the use of microbiological inoculants containing lactic acid bacteria before ensiling them in order to achieve high quality silages (Dinić *et al.*, 2010b; Tyrolova and Vyborna, 2008; Kung *et al.*, 1984).

Despite lucerne being high in animal nutritive value, its growth rate is adversely affected by cool weather, which may reduce the amount of yield to be harvested (White and Lucas, 1990). The feeding of ruminant livestock on forage crops has a detrimental effect on the environment, in terms of enteric methane emission (Mirzaei-Aghsaghali and Maheri-Sis, 2011). Ruminant animals' enteric methane emission is estimated at 80 million metric tonnes per year (Beauchemin *et al.*, 2007). Enteric methane emission in ruminant livestock is a normal process of reducing hydrogen concentration in the rumen, which results from rumen anaerobic digestion (Dewhurst *et al.*, 2009). Methane is a potent greenhouse gas with a radiative potential of 25 times more than that of carbon dioxide (Sejian *et al.*, 2010). The earth's atmospheric accumulation of greenhouse gases such as carbon dioxide (CO₂), nitrous oxide (N₂O) and methane (CH₄) is contributing to the earth's surface temperature increase (Moss *et al.*, 2000). The GHG accumulation rate is estimated to increase between 300 and 600 ppm annually on account of the effects of human activities on carbon and nitrogen cycles (Desjardins *et al.*, 2001). The increased use of forages in ruminant animals' diet

increases enteric methane emissions as the rumen microorganisms have more fibre to digest. Therefore one of the methane emission reduction strategies in ruminants is the feeding of these animals with more cereals or grains. Dairy cows total mixed ration rich in cereals or grains results in reduced enteric methane emission because of increased ruminal digestion and fermentation which results reduced time for the association between methanogens and digesta. This results in reduced enteric methane emission but the effects on whole system carbon footprint depends on the nature, processing and location of feeds.

However, the feeding of ruminant livestock, such as dairy cattle, with high quality legume forages like lucerne has been proven to reduce methane emission because of high digestibility and increased rumen passage rate (Dewhurst *et al.*, 2009). The inclusion of lucerne, (*Medicago sativa sativa.*), as a legume forage in dairy cows' diet increases their performance characteristics in terms of milk production, resulting in reduced enteric methane emission per unit of milk production.

1.2 Lucerne, [*Medicago sativa sativa*]

Lucerne, *Medicago sativa sativa.*, is a high yield perennial nutritious forage legume that grows in different climates all over the world (Lancefield *et al.*, 2009). Lucerne grows well in both tropical and temperate climates and it survives in frosty winters (McDonald *et al.*, 2003). Huyghe (2003) stated (in Annicchiarico *et al.*, 2010) that lucerne is a forage crop of huge importance because of its contribution to sustainable agriculture and its higher productivity of feed proteins per unit area compared to other forages or grain legumes. It is a drought resistant perennial forage legume that can yield high quality forage in times of little rainfall (McDonald *et al.*, 2003). This is because lucerne has a deep root system with a straight taproot that can cover a depth of more than 15 m (Jasjeet *et al.*, 2011; Kokate, 1990). In cold winters, lucerne becomes dormant and when summer approaches it re-grows by using the nutrients reserved in the roots (Zanin, 1998). In areas like California and Egypt 8-10 harvests are annually realised through irrigation. When lucerne is

harvested by cutting, the plant regrows using the root nutrient reserves (Zanin, 1998). In Europe, lucerne grows well from March to October and can be harvested every 40 days depending on temperature (Zanin, 1998).

1.2.1 Lucerne species

There are two species of lucerne:

1. *Medicago sativa sativa* encompasses the most commonly grown lucerne varieties and have purple flowers, taproots that reach up to a depth of 10 m, grows well in hot climates and does not tolerate cold winters. It originated from the Middle East and diversified into the Mediterranean region (Julier *et al.*, 1995).

2. *Medicago falcata*, is a native species in South East England with yellow flowers, has a prostrate growth, fasciculate roots and can be grown in frosty areas with low nutritional requirements. It furthermore has sickle-shaped pods and has winter dormancy. It originated from Central Asia and has spread throughout in northern Eurasia (Julier *et al.*, 1995; Frame, 1998).

1.2.2 The origin of lucerne

The origin of lucerne can be traced back to Middle East regions such as Iran, Turkmenistan and Caucasus in the 20th century BC, as archaeologically evidenced, and in Babylon in around 7th century BC, with evidence from written history (Yuegao and Cash, 2009). It arrived in Spain and the near East from Iran in the 11th century AD and arrived in France and Holland in the 16th century AD (Yuegao and Cash, 2009). In New South Wales, lucerne started being cultivated prior to the 18th century and was soon being cultivated in many parts of Australia (McDonald *et al.* 2003). Lucerne was first cultivated in the UK in the 17th century AD (Yuegao and Cash, 2009). The crop made its way to Europe and then to North and South America. In Mexico and Peru, lucerne was first cultivated by Spaniards and Portuguese colonialists who ruled America in the 16th century AD and they introduced

the cultivation of lucerne in most parts of South America (Yuegao and Cash, 2009).

Lucerne is grown all over the world on an estimated 32 million hectares of land (Yuegao and Cash, 2009). The major lucerne producing areas in the world are North America with 11.9 million ha and South America with 7.0 million ha, representing 41% and 23% of hectares, respectively (Yuegao and Cash, 2009). In Europe, 7.12 million hectares of lucerne is grown, representing 25% of lucerne growing areas, and in Asia, 2.23 million ha of lucerne is cultivated representing 8.0% of lucerne growing hectarage, followed by Africa (2%) and Oceania (1%) (Yuegao and Cash, 2009). Table 1-1 below summarises the world lucerne growing regions and area of land under lucerne cultivation.

Table 1-1: Area of lucerne grown (in ha) in different countries

Country	Lucerne grown (ha x 10 ⁶)
North America	11.90
South America	7.00
Europe	7.12
Asia	2.23
Argentina	6.90
Canada	2.00
Russia	1.80
China	1.30
Italy	1.30
Africa	0.64
Oceania	0.32

Adapted from Yuegao and Cash (2009)

1.2.3 Lucerne growing in the UK

Phipps *et al.* (1997) reported that little lucerne has been grown in the UK because farmers consider it as difficult to grow and conserve due to weed control and conservation/ensiling problems, and from their survey on the amount of lucerne grown in the UK, they found that by 1997 only 309 ha of land were under lucerne cultivation. However, this reported hectarage is

open to question when compared with other studies for example Sheldrick *et al.* (1995). They reported that 20,000 ha of lucerne is grown in the UK. This figure is almost 40% less than the total amount of lucerne grown in the 1950s (45000 ha) (Sheldrick *et al.*, 1995). In the UK, there are about 600,000 ha of land suitable for the growth of lucerne with 200,000 ha of potential land suitable for rotational lucerne growing (Sheldrick *et al.*, 1995).

1.2.4 Lucerne varieties

There are about 30 varieties of lucerne (*Medicago sativa sativa*, L.) available with different disease and pest resistance characteristics, which should be considered when choosing them for cultivation (McDonald *et al.*, 2003). There were six main varieties of lucerne in the UK in 1995, which are classified with reference to their resistance to diseases (Sheldrick *et al.*, 1995). These lucerne varieties include Boreal and Rival which were introduced in the United Kingdom from the United States of America (Sheldrick *et al.*, 1995). Lucerne forage yield and protein content can be maximised by growing lucerne varieties suitable for the UK climatic conditions (Limagrain, 2011). A more recent variety Marshal has a high dry matter yield, drought resistance and is ideally recommended for the UK conditions (Limagrain, 2011). In Europe and the USA efforts have intensified to improve lucerne forage quality and its resistance to a number of pests (Sheldrick *et al.*, 1995). Boreal and Rival varieties have higher disease and pest resistance in contrast with the other four varieties presented in Table 1-2 (Sheldrick *et al.*, 1995).

Table 1-2: Varieties of lucerne available in UK

	Europe	Vertus	Euver	Vela	Boreal	Rival
Yields (% of Europe)	100	97	99	101	96	100
Resistance to Verticillium wilt	3	7	4	7	6	7
Eelworm resistance	4	7	5	3	7	5
Lodging(crop falling over when mature)	7	4	5	4	4*	4*

* lodging of these varieties can be controlled by earlier cutting,

Scale 9= good resistance to pests/ lodging, 1= lowest resistance to pest/ lodging

Source: Sheldrick *et al.*, (1995)

1.2.5 Lucerne growing conditions

Lucerne can be grown in both loam soils with good drainage and sandy loam soil mixed with clay soils. It is necessary to provide drainage systems in clay soils to prevent water logging. It also grows well in rich alluvial loam, or sandy soils with a mixture of clay, rich in calcium, phosphorus and potash resulting in high yields (McDonald *et al.*, 2003; Kokate, 1990). Lucerne is relatively resistant to alkaline soils but high soil alkalinity impairs its productivity (Kokate, 1990). However, established lucerne stands adapt to alkaline soils fairly well while young lucerne forages which have just germinated do not survive in alkaline environments (McDonald *et al.*, 2003). This type of adaptation makes it capable of reclaiming slightly saline areas by lowering the water tables (McDonald *et al.*, 2003).

However, although lucerne grows well in a variety of soils, such as deep well drained soils of medium to light texture (Kokate, 1990), it does not perform well in acidic soils especially where the top 10cm has a pH of less than 5.2 (McDonald *et al.*, 2003). However, liming is one of the most efficient and

prevailing practices to correct soil acidity and improve lucerne yields (Adonis and Fageira, 2010; McDonald *et al.*, 2003). The soil should have very low levels of exchangeable aluminium, usually less than 5 per cent (McDonald *et al.*, 2003). On both moderately and very acid soils where aluminium toxicity exists, more than 2.5 t/ha of lime may be applied (McDonald *et al.*, 2003). This is because acid soils usually have less calcium and molybdenum, meaning nodulation and root growth are impaired (McDonald *et al.*, 2003). Lucerne can be grown in dry land conditions where the average annual rainfall is about 850 mm with moderate to high frost tolerance and can survive long drought conditions but is unproductive in very dry environments (Kokate, 1990; Thawana, 2008; McDonald *et al.*, 2003). When lucerne is grown in waterlogged areas with high temperatures, the plants either die or regrowth is weakened (McDonald *et al.*, 2003). It normally does not survive in wet and muddy areas, such as impermeable clay subsoils in valleys which are often flooded with water (McDonald *et al.*, 2003).

The ideal temperatures for the growth of lucerne is 25⁰ C and high production can occur between 10-30⁰ C, with yield productions significantly dropping when the temperature is below 10⁰ C (McDonald *et al.*, 2003). Mackenzie *et al.* (1988) stated (in Frame, 1998) that lucerne is a frost resistant forage because from 10⁰ C, plant hardening occurs and increases with declining environmental temperatures, up to -1 and -2⁰ C. Barnes and Sheaffer (1995) reported (in Frame, 1998) that lucerne has survived in cold and hot temperatures of -25⁰ C and above 50⁰ C in Russia and California respectively. Although lucerne has been described as frost tolerant forage, considerable leaf damage on actively growing plants may occur especially when the plant was not conditioned by a period of low temperatures prior to the occurrence of the frost (McDonald *et al.*, 2003).

Lucerne is a perennial crop and it grows for a number of years, hence the required nutrients for its growth and productivity must be applied prior to seedbed preparation. It requires a lot of nutrients during its growth, therefore the application of farm organic manure should be done at the rate of 25 tonnes per hectare prior to the preparation of the seedbed (Kokate, 1990).

When the soil pH is below 6.5, lime application should be done at least between three and six months prior to lucerne sowing. In sandy soil with less than 15% clay, for the lucerne stand to produce a high yield with a high degree of shoot re-growth after cutting, less than 100 kg/ha of potash should be applied during planting (Thawana, 2008). Furthermore, another 100 kg/ha of potash fertilizer should be applied on the soil surface as top dressing (Thawana, 2008). McDonald *et al.* (2003) recommends that in soils deficient in phosphates, up to 30 kg/ha of phosphates should be applied (375 kg/ha of single superphosphates or equivalent) in areas with heavy precipitation, and 10 kg/ha should be applied in areas which receive limited amount of rainfall. When cultivating lucerne, sulphur is necessary for plant nitrogen manufacturing and therefore 30-40 kg/ha should be applied at sowing time (Thawana, 2008).

1.2.6 Lucerne stand longevity

Lucerne is a perennial crop that can grow for more than twenty years according to its treatment, climate and variety; however it is usually grown for about six years (Jasjeet *et al.*, 2011; Zanin, 1998). Lucerne rotational growing is practised in Australia with some annually growing crops at a time interval of two-four years and sometimes it is grown for five or more years on the same land (Humphries and Hughes, 2006). The straight taproot, which stretches into the soil to a depth of 8-9 m with an upright height that varies from 60-90 cm, or about 1.0 m make lucerne survive over a long period (Kokate, 1990; Jasjeet *et al.*, 2011).

Lucerne grows well in pure stands and growing it with other crops increases the risk of poor stand establishment (McDonald *et al.*, 2003). This is because other crops or weeds compete with lucerne plants or seedlings for moisture, nutrients and deprive the lucerne plants of sunlight when they are densely planted (McDonald *et al.*, 2003; Sheldrick *et al.*, 1995).

However, lucerne has been grown in mixed stands and Sheaffer *et al.* (1990) reported (in Frame, 1998) that lucerne can be grown in mixed stands only

with grasses that compete favourably for nutrients. In the northern USA, grasses such as smooth brome grass, cocksfoot and reed canary have been grown together with lucerne, although cocksfoot and reed canary grass species outcompete lucerne for nutrients. In Europe, timothy grass (*Phleum pratense*), meadow fescue, cocksfoot and tall fescue (*Festuca arundinacea*) have been grown in mixed stands with lucerne (Frame, 1998). When lucerne is grown with companion crops like timothy and cocksfoot, they should be drilled at no more than 1 kg/ha and 2 kg/ha respectively to avoid competition for essential elements for growth (Limagrain, 2011).

In early stand establishment, lucerne is slow at competing with weeds but it improves with the development of the canopy (Frame, 1998). When lucerne is fully established and is well managed, vigorously growing, dense lucerne stands prevent severe invasion from weeds (Frame, 1998).

1.2.7 Pests and diseases of lucerne and their control

Lucerne is susceptible to different pests attack such as aphids, eelworms, weevils, slugs and diseases like verticillium wilt and crown rot (Limagrain, 2011; Sheldrick *et al.*, 1995). Weevils and slugs usually occur at an early stage of stand establishment and destroy young shoots, and can be controlled by spraying the crop with pyrethroids. Aphid infestation occurs in later stages of lucerne growth and there is not any chemical available for its control (Limagrain, 2011). Manglitz and Ratchliffe (1988) and Leath (1988) reported (in Summers, 1998) that in the USA lucerne anthropods and pests, especially nematodes, and diseases can cause a loss of \$260 million and \$400 million annually. Limited information was available on the effects of pests in the UK. The most important lucerne pests in America are nematodes, which can be classified further as stem nematodes (*Ditylenchus dipsac*), northern root-knot nematodes (*Melodogyne hapla chitwood*) and the southern root-knot nematodes (*M. Incognita*) (Kofiod and White) Chitwood, and these pests can be controlled by planting resistant lucerne varieties (Summers, 1998). Summers (1998) summarised the lucerne pests and their control measures as shown in Table 1-3.

Table 1-3: Summary of the strategies for the management of major pests of lucerne forage

Pest	Cultivar resistance	Biological control	Cultural Control	Chemical control
Stem nematodes	Yes	No	Delay irrigation, Sanitation	None
Root-knot nematodes				
NRKN	Yes	No	Avoid heavily infested fields, Sanitation	None
SRKN	Yes	No	Avoid heavily infested fields, Sanitation	None
Lesion-Nematodes	No	No	Fallow, rotation	Limited

Adapted from Summers, (1998), NRKN= northern root-knot nematodes, SRKN= southern root-knot nematodes

Eelworms (*Ditylenchus dipsaci*) occur in waterlogged soils and are a source of persistent problems in lucerne stands. Eelworms can be prevented by planting lucerne varieties that are resistant to these pests and using fumigated lucerne seeds (Limagrain, 2011). Lucerne weevils, like sitona weevil larvae, slugs and leatherjackets can be controlled by insecticidal spraying such as cypermethrine (Sheldrick *et al.*, 1995) and by the use of slug pellets (Limagrain, 2011).

Verticillium diseases can only be prevented by growing resistant varietal crops because there is no chemical to control them (Limagrain, 2011). Sheldrick *et al.* (1995) stated that verticillium wilt diseases are the main cause of the decrease in lucerne production in the UK. Verticillium wilt causes almost a decline of 50 % of the crop especially in the second harvest of the year (Sheldrick *et al.*, 1995). Verticillium wilt is characterised by botchy yellow leaves and brown markings and leaves normally wilt leaving bare green stems (Sheldrick *et al.*, 1995).

Crown rot diseases are rare but occur after a heavy application with slurry or when wet lucerne fields have been overstocked (Sheldrick *et al.*, 1995).

Leath *et al.* (1988) reported (in Summers, 1998) that anthracnose, which attacks lucerne stems where lesions make stems girdle, is the major lucerne disease in the world caused by *Collectotrichum trifolii*. The lucerne crowns are also attacked by *Collectotrichum trifolii*, resulting in the death of the plant (Summers, 1998). Leath *et al.* (1988) reported (in Summers, 1998) that another disease occurring in lucerne stand is downy mildew, caused by *Peronospora trifoliorum* dBy., which sometimes kills the seedlings, resulting in total stand failures. Elgin *et al.* (1981) reported (in Summers, 1998) that these diseases mostly can be controlled in lucerne fields by growing cultivars with moderate to high levels of disease resistance (see Table 1.2) and this will also result in higher dry matter yields and stand persistence.

Lucerne forage diseases can also be controlled by cultural practices or strategies such as early or delayed harvest, water management, sanitation and crop rotation (Summers, 1998). Field water management by field drainage construction achieves a reduction of the incidence of *Phytophthora* root rot and *Aphanomyces* root rot, which occur in water saturated soils (Summers, 1998).

1.2.8 Nutrient content and its feed value

Lucerne is a high nutritious forage legume with high animal feeding values and about 480 g/kg DM of the weight of the plant consists of leaves (McDonald *et al.*, 2003; Kokate, 1990). It is rich in proteins, minerals and vitamins (Frame, 1998). The animal feeding value of lucerne is mainly defined by the stage of growth at the time of its harvest, as the plants' nutrient content decreases with advancing maturity, resulting in the decrease of the leaf: stem ratio (Frame, 1998; Tyrolova and Vyborna, 2008). Keftassa and Tuveesson (1993) stated (in Frame, 1998) that lucerne fibre proportion increases by 1.6 g/kg of DM daily as the plant grows, thereby increasing the degree of indigestibility especially in the lower fractions of the stems. The nutritive value of lucerne, in terms of digestibility and protein content, decreases with advancing maturity by 0.3-0.5% per day from early flowering to near maturity stage as shown in Table 1-4 (McDonald *et al.*, 2003).

Table 1-4: The effect of stage of maturity on lucerne nutrient quality

Stage of Maturity	Crude Protein g/kg DM	ME (MJ / kg DM)	Digestibility %
Early Vegetative	230	10.4	72.90
Late Vegetative	200	9.9	70.00
Early bloom	180	9.3	66.50
Mid bloom	170	8.9	64.10
Full bloom	150	8.4	61.20

Source: McDonald *et al.* (2003).

Lucerne is a high nutritive value forage in contrast to other typical grasses and cereals which are commonly used in animal feeds, as shown in Table 1-5 (Courtney, 2003).

Table 1-5: The nutrient content of lucerne compared to other typical grasses and cereals

Green Forages	Dry Matter %	ME (MJ / kg DM)	Crude Protein g/kg DM
Lucerne, Full bloom	24	8	150
Ryegrass, Perennial, Mature	30	10	80
Cocksfoot, Mature	32	9	80
Clover, White, Mature	23	9	150
Barley, Post bloom	21	10	50
Grass- dominant pasture, mature	40	5	20
Oats, post bloom	23	10	80

Source: Deborah Courtney, (2003).

Katic *et al.* (2006) stated (in Katic *et al.*, 2009) that crude protein content in lucerne ranges between 18-25 %, with an oil and mineral proportion content of 1.78% and 9.87%, depending on the species being cultivated. Lucerne forages on as fed basis (fresh basis) contain 80.0% water, 5.2 % protein, 0.9 % fat, 3.5 % fibre and 2.4 % ash (Duke, 1983). Duke (1983) further stated that lucerne, on fresh basis, is a source of vitamins A and E with 6.24 mg/g B-carotene, 0.15 mg/g riboflavin and 0.46 mg/g niacin.

Lucerne crude protein content decreases with advances in maturity compared to crude fibre content which increases (Stanacev *et al.*, 2010). Lucerne forages which are cut when the plants are fully flowered have more stem proportion than forages cut at early stages of growth (Tyrolova and

Vyborna, 2008). Although lucerne is high in proteins and metabolisable energy content as indicated in Table 1-5 (Courtney, 2003), the proportion of leaves at the time of harvest and the stage of growth at which it is harvested is a major factor that determines the quality of the crop (Tyrolova and Vyborna, 2008). Lucerne leaves have a higher nutrient content than stems, on a dry matter basis, and on average lucerne forage contains 18-22 % crude protein with lucerne leaves and stems separately containing 26-30 % and 10-12 % crude protein respectively (Arinze *et al.*, 2003).

1.2.9 Antinutritional factors

Despite its high nutrient content in terms of proteins, lucerne leaves contain antinutritional factors such as protease inhibitors, saponins, flavonoids/ flavones, isoflavones, sterols and has a high estrogen activity (Jasjeet *et al.*, 2011; Katic *et al.*, 2009). However, lucerne saponins have been assumed to be degradable by the rumen microbes releasing sugars and aglycon after the glycosidic bond is broken (Sen *et al.* 1998).

1.2.10 Harvesting lucerne

Harvesting conditions can have a major effect on nutrient quality. Lucerne is a versatile forage crop that has been harvested and used as pasture, hay, silage haylage, and baling and green chop (Lancefield *et al.*, 2009; Zanin, 1998). Lucerne can be harvested by removing the foliage every 25 to 45 days during the growing season (Summers, 1998). At harvesting, good quality lucerne usually has 185 g/kg fresh weight crude protein and 625 g/kg total digestible nutrients (TDN) on a as fed basis (Lancefield *et al.*, 2009). When lucerne hay is well managed during cutting/harvesting, it contains high nutritive feed value of metabolisable energy (ME) levels of up to 11 MJ/kg of DM, and crude protein (CP) content of around one fifth of every unit mass of lucerne (McDonald *et al.*, 2003). The hay quality varies with the stage of growth at cutting, the stem to leaf ratio, the hay making techniques, weed proportion, weather damage extent on the crop and the amount of moulds present on the crop (McDonald *et al.*, 2003). Lucerne yields for commercial purposes in Australia range from 10-22 t/ha from six cuts between early

October and late April and, under good management, lucerne yields of 25-27 t/ha, on dry matter bases in the form of hay, have been achieved (McDonald *et al.*, 2003).

Sheldrick *et al.* (1995) reported that annual lucerne hay production in the UK, after stand establishment is in the excess of 10.5 tonnes dry matter (DM) per hectare. The harvested yields in the first two cuts, when flower buds are at two nodes on the main stems, are almost equal. However, for this simple rule to be effective the first cut of the harvest year should be made when 30% of the florets are open, in order to allow adequate root development (Sheldrick *et al.*, 1995). After the first and second cuts, whose yields are similar, the following cuts are lower yielding (Sheldrick *et al.*, 1995).

Table 1-6: The proportion of lucerne harvested at different stages of the season

Cut	% Total Yield
Late May	35
Early July	35
Mid-August	20
Late October / early November	10
	100

Source: Sheldrick *et al.* (1995)

1.2.11 Hay making

Lucerne which is cut at the early flowering stage is high quality; however, frequent harvesting at this stage compromises lucerne longevity (McDonald *et al.*, 2003). The hay is sometimes harvested or cut at 10 % flowering rate (Kokate, 1990). However, the timing of the flower appearance depends on a combination of factors such as soil moisture, temperature, day length and variety. Cutting of lucerne can be done when half of the crown buds are 1.5cm apart, although this is also variable because bud appearance and elongation vary with lucerne variety, growing conditions and past management (McDonald *et al.*, 2003). The harvesting should be done when new shoots are short enough to avoid damaging them with the cutter bar. Lucerne which is cut in the afternoon contains higher nutrient content than that cut in the morning, and hay cutting in cooler conditions should be

avoided because it allows respiration to continue, resulting in the loss of lucerne nutritive value such as WSCs (McDonald *et al.*, 2003).

After harvesting, lucerne should be rapidly dried to prevent continued respiration that depletes WSC, nutrient loss from weathering and microbial plant degradation (McDonald *et al.*, 2003). It should not be overdried to avoid loss of leaves through shattering and the stems becoming brittle, which results in low nutrient quality (Lancefield *et al.*, 2009; McDonald *et al.*, 2003). The leaves have high total digestible nutrient (TDN) in the form of energy and crude protein and therefore leaf loss during cutting and drying should be avoided (Lancefield *et al.*, 2009). The hay should always have a moisture content ranging from around 170-180 g/kg DM and in hot dry conditions; hay baling is done at 210 g/kg DM content (MacDonald *et al.*, 2003). Radović *et al.* (2009) reported that during lucerne harvesting, there is a loss of dry matter ranging from 300-500 g, 50-200 g and 50-70 g/kg DM when the forage is field dried, ensiled and dehydrated (made into hay) respectively. The haymaking process is usually associated with high loss of digestible energy because of increased leaf loss in rain-damaged hay in first cut lucerne (Radović *et al.*, 2009).

1.2.12 Lucerne silage making

In the UK the largest proportion of lucerne growers (66%) conserve it by baling followed by clamping (58%) (Phipps *et al.*, 1997). The main problem in lucerne conservation in the UK is bad weather conditions (cold weather), which result in ineffective wilting of lucerne during drying, thus making it difficult to ensile (Phipps *et al.*, 1997).

High quality lucerne silage can be produced when lucerne forage is rapidly wilted (by crushing the lucerne stems to speed up the loss of water) before ensiling (McDonald *et al.*, 2003; Sheldrick *et al.*, 1995). Lucerne when it is freshly cut from the field has a too low DM content, too high protein and calcium content, and too low sugar content to achieve a high degree of silage fermentation (McDonald *et al.*, 2003; Dinić *et al.*, 2010b). This results

in high buffering capacity against any changes in acidity (McDonald *et al.*, 2003). Wilting removes excess moisture from the forage, concentrating its sugars and allowing effective silage fermentation (McDonald *et al.*, 2003). However despite these short comings, lucerne can be wilted to at least 300 g/kg or 350 g/kg DM to be successfully ensiled (Kung, 2010; Tyrolova and Vyborna, 2008; Dinić *et al.*, 2010a). Depending on the environmental weather conditions, wilting takes 36 hours to achieve the desirable lucerne DM content for ensiling (McDonald *et al.* 2003). However, when lucerne is wilted during high precipitation or high humidity, reduction in the amount of WSCs occurs because of extended respiration, which breaks the stored sugars to carbon dioxide, water and heat, resulting in poor quality silage (Kung, 2010, McDonald *et al.*, 2003). Sheldrick *et al.* (1995) reported that when lucerne with moisture content above 70 % is ensiled by clamping, 20 litres of molasses or 6 litres of formic acid per unit tonne should be added to enhance fermentation. The silage making in big bales should be done after the lucerne has been wilted to 400-600 g/kg dry matter content before wrapping them in polythene bags and mature lignified lucerne forages should be chopped to prevent piercing the polythene bags, although pre-chopping adversely affects fermentation processes (Sheldrick *et al.*, 1995).

Sheldrick *et al.* (1995) reported that different methods of conserving or harvesting lucerne have different detrimental effects on the nutrient (quality) of lucerne forage as shown in Table 1-7.

Table 1-7: The effects of different methods of conserving lucerne on quality

	Silage	Field dried hay	Barn dried hay	Dehydrated pellets
Dry Matter (DM %)	30.00	85.00	85.00	90.00
pH	4.50	-	-	-
D value	63.00	55.00	60.00	58.00
CP (g/kg DM)	200.00	170.00	200.00	180.00
Degradability	75.00	65.00	60.00	50.00

Source: Sheldrick *et al.* (1995),

D value= Digestible value

1.3 Silage making in dairy farming

Silage making, for both dairy and beef cattle farmers, is aimed at producing good quality, high energy and protein-rich conserved feed (Jatkauskas and Vrotniankiene, 2009). Ensiling of both legume and grass forages is one of the major means of preserving forages for livestock consumption (Muck, 1996; Jones *et al.*, 1991a; Halling and Scholefield, 2001). It is a simple technique of conserving forages by compression, followed by airtight sealing (Danner *et al.*, 2003). Ensiling conserves forage biomass and its by-products, together with their nutrients, by using either chemical or biological additives which rapidly reduce the forages pH to a low final pH (Dinić *et al.*, 2010b; Jones *et al.*, 1991a).

In the fermentation of forage crops, hemicellulose are degraded by plant enzymes, some microbes, and chemical or acid hydrolysis (Morrison, 1979; Dewar *et al.*, 1963) resulting in reduced forage silage neutral detergent fibre (NDF) content in the form of carbohydrates compared to the initial herbage content (Chestnut *et al.*, 1988).

McDonald *et al.* (1991) stated (in Jatkauskas and Vrotniankiene, 2009) that to achieve high quality silages ensiling regulations must be observed, especially when ensiling herbages with high buffering capacity like lucerne. The feeding value of lucerne silages for dairy cattle is influenced by lucerne characteristics, the stage of growth of the crop at the time of ensiling, and the degree and type of fermentation achieved within the silo (Jatkauskas and Vrotniankiene, 2009).

1.3.1 Ensiling Process

The ensiling process of forages in an ideal situation takes 3 to 4 weeks to be completed and reach a stable phase (Kung and Der Bedrosian, 2010). This process involves four major biological phases which are: pre-sealing/respiration, active fermentation, stable phase and silage feed out (Barnhart and Nadeau, 2008) as shown in Table 1-8.

Table 1-8: Biological processes of ensiling

	Pre-seal	Active fermentation	Stable phase	Feedout
Plant respiration				
Protein degradation				
Yeasts				
Molds				
Acetic acid bacteria				
Lactic acid bacteria				
Clostridium bacteria				

Source: R.E. Muck and R.E. Pitt, 1993, (in Barnhart and Nadeau, 2008), Ensiling Process and Additives, Iowa State University) "Ensiling and its effect on crop quality," p. 57. In Proceedings of the National Silage Production Conference, Syracuse, NY. 23-25 Feb. 1993. NRAES Cooperative Extension, Ithaca, NY.

1.3.3 Pre-sealing / Respiration

This involves the chopping, filling and packing of the forages in which plant and microorganism respiration dominates, causing nutrient changes and losses from the chopped forages (Barnhart and Nadeau, 2008). During respiration there is a reduction or depletion of the plant WSCs (sugars) which are used to produce carbon dioxide, water and heat in the presence of oxygen (Kung, 2010). The depletion of water soluble carbohydrates by extended respiration results in reduced lactic acid production during ensiling (Barnhart and Nadeau, 2008). The heat produced during respiration raises the temperature of the silo resulting in the Maillard reaction which decreases silage digestibility (Barnhart and Nadeau, 2008).

Respiration in silage is reduced by wilting the forages to at least 30-35 % DM to reduce air trapped in the forages during packing (Kung, 2010; Barnhart and Nadeau, 2008). Ensiling forage crops at dry matter content lower than 30 % results in silage pH values higher than 4.6-4.8 because of clostridial fermentation, whereas ensiling forages at more than 45-55 % DM content inhibits the growth of lactic acid bacteria (LAB) resulting in low lactic acid production (Kung, 2010).

1.3.4 Active Fermentation

This phase occurs under anaerobic conditions and is dominated by the growth of lactic acid bacteria (LAB). It usually lasts from one to four weeks resulting in the decrease of silage pH to around 4.0 (Barnhart and Nadeau, 2008; Stefanie *et al.*, 2000). The main active microbes in this phase are homo- and hetero-LAB and LAB, are the epiphytic bacteria of the forages (Stefanie *et al.*, 2000). However, hetero LAB are not desirable in this phase, because they produce acetic acid and ethanol which reduce the silage forage DM (Barnhart and Nadeau, 2008; Kung, 2010). The amount of forage sugar, DM content, sugar composition and LAB osmotolerance determine the ability of the LAB to compete with the undesirable microbes like enterobacteria and clostridia (Stefanie *et al.*, 2000).

1.3.5 Proteolysis

During ensiling, in the first days of the active fermentation stage when the pH is between 5.5 and 6.0, protein is broken down to non-protein nitrogen (NPN) by proteases enzymes in the forages (Muck, 1996; Barnhart and Nadeau, 2008; Kung, 2010). In this process, the proteins are decomposed to peptides and amino acids which are further converted to NH_3 and amines by microbial activities (Muck, 1988; Muck, 1996). In ensiled lucerne 850 g/kg of the total forage N may result in being NPN because of the conversion of crude protein nitrogen to NH_3 (Muck, 1987). Proteolysis during ensiling is inhibited by increasing the rate of pH decline (Marshall *et al.*, 1993) but because of the partial fermentation that occurs in high quality forages like lucerne the rate of pH decline is slow. This encourages the growth of harmful microbes like clostridia (Barnhart and Nadeau, 2008). There are two types of clostridia bacteria; *saccharolytic* and *proteolytic*. *Saccharolytic* bacteria convert carbohydrates to butyric acid, carbon dioxide and hydrogen whereas *proteolytic* bacteria convert amino acids to organic acids, carbon dioxide, ammonia and amines respectively (Muck, 1988; Carpintero *et al.*, 1969). Clostridia bacteria and yeasts convert lactic acid to foul smelling butyric acid and produce ammonia from plant protein (Barnhart and Nadeau, 2008). Clostridia bacteria increase the concentration of butyric acid and ammonium

(NH_4 > 10% of plant nitrogen) and reduce lactic acid concentration, resulting in silage with a pH above 5.0 (Barnhart and Nadeau, 2008). The high concentration of butyric acid increases both the silage acid detergent fibre (ADF) and neutral detergent fibre (NDF) because of the degradation of soluble nutrients in silage and the silage may have some amines (Kung, 2010).

Good silage fermentation is characterised by a dry matter loss of between 100 and 120 g/kg DM of the total silage whereas poor fermentation is signified by a dry matter loss of more than 200 g/kg DM because of effluent run off, oxidation and loss of volatile organic matter from the ensiled forages (Barnhart and Nadeau, 2008; Kung, 2010).

1.3.6 Enterobacteria and silage carbohydrate losses

Enterobacteria are anaerobic bacteria and mainly ferment sugars, producing acetic acid resulting in both high DM and energy losses during silage active fermentation process (Muck, 1996). The loss in total silage DM is mainly on account of high decomposition of highly soluble nutrients such as proteins and carbohydrates (Wattiaux, 1999). The loss of highly soluble nutrients such as proteins and carbohydrates increases the proportions of fibre content in the form of both ADF and NDF (Wattiaux, 1999).

1.3.7 Stable phase

This occurs when LAB has used up all the sugar in the silage, or when the pH of the silage has decreased to 4.0-4.2, and little or no biological activity occurs as long as air is prevented from entering the silo (Barnhart and Nadeau, 2008; Stefanie *et al.*, 2000). At this stage, the fermentation process is assumed to have stopped and the growth of undesirable bacteria is inhibited (Barnhart and Nadeau, 2008). However, some acid-resistant proteases and carbohydrases, together with acidic bacteria like *Lactobacillus buchneri*, *bacilli* and clostridia, survive in either a less active state or as spores (Stefanie *et al.*, 2000).

1.3.8 Feed out phase or aerobic spoilage phase

This is when the silage is opened and exposed to air for feeding. During this stage, moulds, aerobic bacteria such as the *bacilli* species and yeast, metabolise the lactic acid and residual sugars in the silage, producing carbon dioxide, water and heat, resulting in increasing pH levels (Barnhart and Nadeau, 2008; Stefanie *et al.*, 2000; Woolford, 1984).

The high pH levels allow microorganisms that were inhibited to grow, consequently resulting in silage spoilage (Barnhart and Nadeau, 2008; Stefanie *et al.*, 2000; Woolford, 1984). The dry matter content and the energy value of the silage are reduced, because of the degradation of WSC and organic acid, and the volatile basic nitrogen concentration is increased resulting in reduced feed silage palatability and voluntary intake in dairy cows (Jatkauskas and Vrotniankiene, 2009). Yeast species such as lactic acid utilising *Candida* and *Hansenula* are the most active during feedout and they cause silage aerobic stability problems (Woolford, 1984). The increase in silage temperature to about 60°C, especially in hot countries, produces Maillard products because of Maillard reaction (Muck, 1988). Maillard reaction is the reaction between carbohydrates and amino acids, especially lysine, when silage is heated up in the silo, resulting in brown to black plastic substances or charcoal-like substances (Theander, 1980).

The rise in the silo temperature results in the loss of 1.5-4.5% DM per day from the ensiled silages (Stefanie *et al.*, 2000, Barnhart and Nadeau, 2008). Honig and Woolford (1980) stated (in Stefanie *et al.*, 2000) that the DM loss that takes place during feedout is equivalent to the DM loss that occurs when silages are kept over a long period of time in airtight silos.

Lactic acid producing bacteria often includes homofermentative microbes such as *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Pediococcus cerevisiae* and *Pediococcus acidilactic* which produce lactic acid (Combs and Hoffman, 2001). These bacteria are effective in decreasing the pH levels of silages, reducing silage dry matter loss and improving animal performance but LAB inoculants decrease aerobic stability of silages during feedout (Combs and Hoffman, 2001). Silage aerobic stability decreases because the lactic acid which is produced by these microbes is easily oxidised by yeasts

when the silage is exposed to air (oxygen) (Combs and Hoffman, 2001). Airtight silos and the removal of at least 5.08cm of silage per day in winter and 10.16 cm of silage per day in summer during feedout reduce silage aerobic deterioration (Barnhart and Nadeau, 2008).

Kung (2010) summarised the three main factors that are important in forage ensiling to achieve good quality silage with improved aerobic stability as follows:

- (1) Complete removal of air from the silo, which can be accomplished by good silo packing, good length of forage chops, low moisture content and rapid sealing of the silo.
- (2) Rapid pH decrease and the extent of silage fermentation achieved in the silo with respect to the initial decrease of silage pH.
- (3) Air exclusion from the ensiling silo and inhibition of yeast growth during silage storage and feedout to avoid aerobic deterioration of the silages. Kung (2010) graphically presented these three silage fermentation factors as shown below.

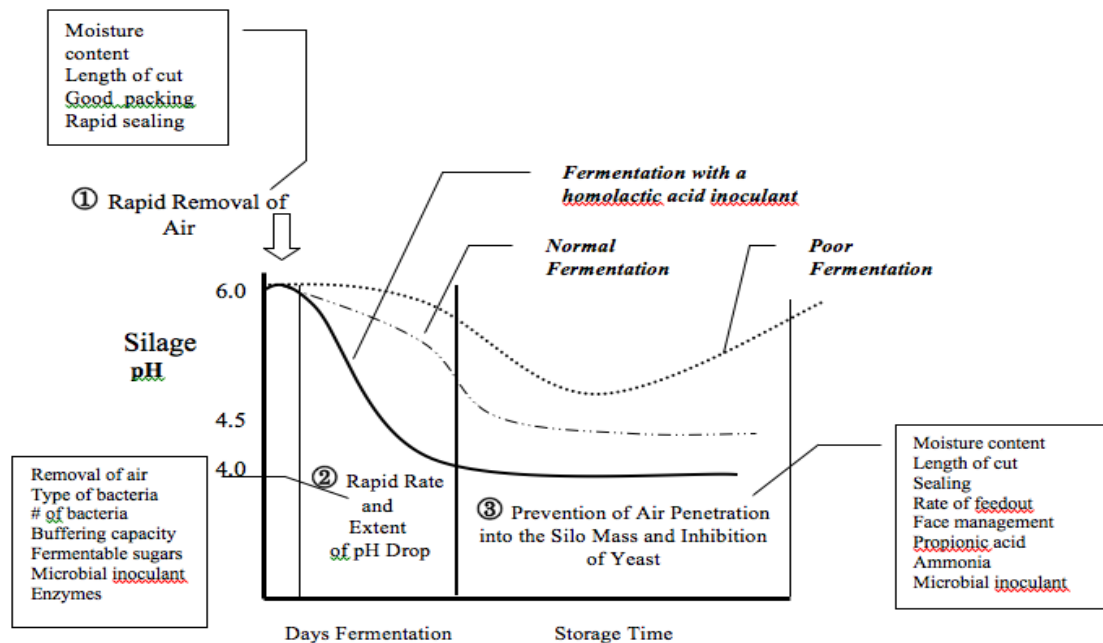


Figure 1-1: The effect of delayed filling on (A) water soluble carbohydrates and (B) dry matter (DM) loss in maize silage. Hirsh and Kung, University of Delaware, unpublished data

Source: Kung (2010). Understanding the Biology of Silage Preservation to Maximise Quality and Protect the environment. 2010 California Alfalfa and Forage Symposium and corn/cereal Silage Conference.

1.3.9 Silage fermentation by-products, their uses and significance in final product quality

Lactic acid is responsible for the decrease in silage pH during ensiling and lactic acid fermentation reduces forage dry matter and energy loss (Kung, 2010). The lactic acid, which is produced during silage ensiling, is consumed together with the silage and is converted to propionic acid by the rumen microbes, such as *Selenomonas ruminantium*, *Megasphaera elsdenii* and *Propionibacterium* (Kung, 2010). The propionic acid is turned into glucose after being absorbed from the rumen by the cow's liver. In good silage, the concentration of lactic acid should be between 650 and 700 g/kg DM of the total amount of silage acids. However, higher amount of lactic acid causes acidosis resulting in loss of appetite in cows (Kung, 2010).

High acetic acid concentration in silages between 30 and 40 g/kg of DM is a result of ensiling forages at low DM content, usually between 250 and 300 g/kg DM, high buffering capacity, loose packing of the silages, and slow silo filling (Kung, 2010). The high concentrations of acetic acid results in low forage DM and energy recovery from the fermentation process (Kung, 2010; Driehuis *et al.*, 1999).

Acetic acid, when consumed by the cows, assists in increasing milk fat production and metabolisation of energy in cows. Acetic acid production is high in silages treated with *Lactobacillus buchneri* (Kung, 2010) and no decrease in dry matter intake by cows has been noticed (Kung *et al.*, 2003; Driehuis *et al.*, 1999).

In well fermented silages propionic acid is rarely found and in poorly fermented silages, propionic acid is found in the ranges of 0.3 to 0.5% because of the effects of some strains of clostridia bacteria (Kung, 2010).

High concentration of ethanol is a result of high yeast metabolism on WSCs, which causes high DM loss and a decrease in aerobic stability during feedout. Ethanol has a lot of energy and is used in glucose formation when consumed by animals but high levels of this silage by-product (between 4-5%) cause off-flavoured milk and wobbling in cows because of the alcohol in the ethanol (Kung, 2010).

Silages with butyric acid concentration greater than 50 g/kg of silage DM content indicate clostridial fermentation and the butyric acids cause ketosis in lactating cows when consumed together with the silage (Kung, 2010).

When consumed by cows, butyric acid is converted to betahydroxy butyrate and acetoacetate increasing the amount of ketones in the animal's body (Kung, 2010). High concentration of butyric acid in silages is associated with high pH, high proliferation of moulds and spores of *Bacillus spp.* and *clostridia tyrobutyricum* (Jonsson, 1991).

In silage fermentation, high levels of ammonia, from 12 to 15 % of the crude protein (CP), indicate a great extent of protein degradation because of the slow decrease in silage pH with a high proliferation of clostridia bacteria and enterobacteria (Kung, 2010). High levels of NH_3 are expected in wet silages with a DM content of less than 300 g/kg because of clostridial growth (Kung, 2010).

Kung (2010) reported that high volumes of $\text{NH}_3\text{-N}$, acetic acid, butyric acids and ethanol in the final silage product indicates poor fermentation and can be used to make some precise assumptions about the kind of micro-organisms that dominated the fermentation process.

1.3.10 Biological / bacterial inoculants

Biological and chemical inoculants are used in forage fermentation to improve the silage fermentation characteristics (Dinić *et al.*, 2010b). Biological inoculants undergo complete degradation during ensiling with no harmful biological residues to the health of the animals and its products compared to chemical inoculants (Dinić *et al.*, 2010b; Dinić *et al.*, 2005). The increasing number of commercial biological inoculants/ additives contains both selected strains of LAB, which are normally applied at 10^5 colony forming units (cfu) per gram of fresh forage, and cellulolytic supplements or cellulolytic enzymes (Dinić *et al.*, 2010b; Barnhart and Nadeau, 2008).

Some biological inoculants, being enhancers of lactic acid fermentation, contain enzyme supplements with cellulase, hemicellulase, amylases, pectinase and ligninase (Dinić *et al.*, 2010b). Dordejević *et al.* (1998a) stated (in Dinić *et al.*, 2010b) that these enzymes decompose the crude cellulose, and the final products are carbohydrates with a lower molecular mass, which are used as food or substrate for the lactic acid bacterial activity. The cellulase enzymes are particularly useful when ensiling lucerne forages which contain insufficient amount of fermentable

Lactic Acid Bacteria (LAB) used in biological inoculants are divided into two physiological groups of homofermentative and heterofermentative. The

heterofermentative LAB degrades some proportions of fructose to mannitol, releasing carbon dioxide, alongside lactic acid and acetic acid production when fructose is the only source of WSCs (Tyrolova and Vyborna, 2008). McDonald *et al.* (1987) stated (in Tyrolova and Vyborna, 2008) that in silage fermentation with homofermentative LAB, 2 moles of lactic acid are released from any fermentable hexose and fructose.

Most biological inoculants contain homofermentative LAB species of *Lactobacillus plantarum*, *Enterococcus*, *Lactococcus*, *Streptococcus* and *Pediococcus* species (Dinić *et al.*, 2005; Driehuis *et al.*, 1999). McDonald (1981) stated (in Muck 1988) that these microbes grow in both aerobic and anaerobic conditions but under aerobic conditions they produce less lactic acid compared to carbon dioxide and water. The anaerobic conditions are favourable for both the growth of many microorganisms and for the production of acids (Muck, 1988). LAB use both monosaccharides and disaccharides as substrates for fermentation (Muck, 1988).

The performance of biological inoculants over a range of forage crops, forage moisture, silo temperatures and aerobic stability during feedout have been improved by using more than one species of microbial strains in one additive (Muck, 1996). For instance, strains of microbes which are capable of growing at different pH are put in one inoculant to achieve a rapid fermentation over a range of pH in the silage (Muck, 1996).

Most microbiological inoculants use *Lactobacillus plantarum* strains with different specific characteristics (Heron and Owen, 2010). A silage additive containing *Lactobacillus plantarum* MTD-1 is active over both a wide range of pH and DM proportions and does not contain any enzymes (Heron and Owen, 2010). In the ensiling of legume forages at DM percentages between 25 and 40 both Ecosyl 100 inoculant (containing *Lactobacillus plantarum* MTD-1 strains) and formic acid performed equally well at 40 % DM, with formic acid performing slightly better than LP MTD-1 at 25% DM (Heron and Owen, 2010). Whiter and Kung (2001) observed both enhanced pH decline and lucerne quality fermentation in 300 g and 540 g/kg DM samples treated

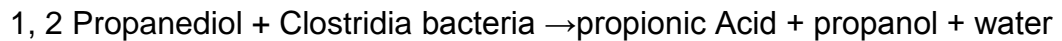
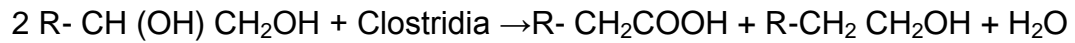
with single strain of both dry and liquid *Lactobacillus plantarum* MTD-1 compared to the control silages on laboratory scale fermentation.

1.3.11 The effect of biological inoculants on silage aerobic stability

The stability of silage when exposed to air is referred to as anaerobic stability and can be measured as the time the silage takes to heat up during feedout. Aerobic stability is hard to achieve, even in well managed silage fermentation (Muck and Hintz, 2003). Microbial additives containing LAB cause a reduction in silage aerobic stability because high levels of lactic acid are not effective fungicides (Jatkauskas and Vrotniankiene, 2009; Muck and Hintz, 2003). The homofermentative LAB are capable of reducing the final pH of silages and reduce proteolysis (Driehuis *et al.*, 1997) but homofermentative LAB impairs silage aerobic stability (Jatkauskas and Vrotniankiene, 2009). Aerobic stability of silages is reduced when only homofermentative LAB are used as inoculants, because the lactic acid produced by these microbes, though in excess, is easily oxidised by yeasts and other microorganisms in the presence of air (Kung, 2010). A number of chemicals have been tried in inhibiting aerobic spoilage, but they resulted in DM and nutritive value reduction after the silage was exposed to air (Kung, 2010).

An alternative biological additive has been prepared from *Lactobacillus buchneri* (Kung, 2010; Nishino and Touno, 2005; Jatkauskas and Vrotniankiene, 2009). *Lactobacillus buchneri* is a heterofermentative LAB which produces acetic acid, resulting in the inhibition of the rapid growth of yeast and improving silage aerobic stability (Nishino and Touno, 2005; Jatkauskas and Vrotniankiene, 2009; Kung, 2010). Silage inoculants containing heterofermentative LAB produce more acetic acid as well as 1, 2-propanediol acid and less lactic acid (Danner *et al.*, 2003).

However, Gaston and Stadtman (1963) stated that 1, 2 propanediol is a media or substrate for the growth of clostridia bacteria which convert 1, 2-propanediol to propionic acid by the following mechanism:



Chemical equation 1. Source: Toraya *et al.*, (1979).

In silage fermentation propionic acid concentration ranges from 0.1-0.2% and is high in silages fermented at DM less than 250 g/kg (Kung, 2010). In fermentation of forages with ideal DM content of 35-45 %, propionic acid is almost negligible (Kung, 2010). However, propionic acid in silages enhances aerobic stability because it is antifungal (Dinić *et al.*, 2010b). Woolford (1979) stated (in Davies *et al.*, 2007) that poorly fermented silages with high concentration of volatile fatty acids, butyric acid and ammonia have higher aerobic stability than well-fermented silages because these VFAs, together with ammonia, are effective silage preservers. Therefore, the presence of propionic acid in final silage fermentation could be either because of poor fermentation or slurry contamination and this scenario needs to be carefully examined.

In summary, an effective silage biological inoculant should contain both homo and hetero fermentative LAB. This is because it will both decrease the silage pH to the level desirable for quality silage production (pH 4.0) and maintain the aerobic stability of the silage during feedout.

1.4 Ensiling of lucerne

Lucerne has proved hard to conserve or ensile under UK conditions (Phipps *et al.*, 1997) but silage is often the only option of preserving lucerne, instead of haymaking, because of high humidity. Therefore, high quality lucerne silage can be achieved by inoculating the lucerne forages with biological additives after the lucerne has been wilted to at least 300 g/kg DM.

The ensiling starter cultures, such as biological inoculants, are effective when the WSCs content in forages is 30 g/kg DM (Szusc *et al.*, 2011). The majority of forages are easily ensiled to produce quality silages because they have high WSC content and low protein content (Dinić *et al.*, 2010b).

However, lucerne is characterised by low WSC and high protein content, which renders it with a high buffering capacity (Dinić *et al.*, 2010b; Muck and Hintz, 2003). Lucerne has low epiphytic LAB (Muck, 1989) and this increases the lag time for the pH to decrease during ensiling (Pitt *et al.*, 1985).

The high buffering capacity renders lucerne hard to ensile because a low amount of lactic acid is produced in the first days of ensiling (Dinić *et al.*, 2010b). The lactic acid produced is insufficient to decrease the pH enough for butyric fermentation to stop; as a result the lactic acid content is surpassed by (converted to) butyric acid (Dinić *et al.*, 2010b; Tyrolova and Vyborna, 2008). This accelerates the decomposition of proteins and amino acids producing ammonia (NH₃) in the silo as by-products, as well as increasing the pH of the silage and the silage spoils (Dinić *et al.*, 2010b; Tyrolova and Vyborna, 2008).

1.4.1 Forage quality characteristics for ensiling

It is documented that the decrease in pH in the lactic acid fermentation process in forages is influenced by the amount of carbohydrates in the green mass and the buffering capacity (BC) of the forages (Dinić *et al.*, 2010b). The BC of any forage is defined as the amount of lactic acid required to

decrease the silage mass to pH 4.0, measured in milli-equivalent (meq) of lactic acid per 100 g DM (Dinić *et al.*, 2010b; Muck, 1988). The proportion of substrates necessary for complete silage fermentation is determined by the BC and the DM content of the forages (Muck, 1988).

The ideal quality of plants/forages for silage fermentation can be defined by the ratio of carbohydrates (S-sugars) and BC (Dinić *et al.*, 2010b). In silage fermentation only half of the WSC are converted to lactic acid, showing that the carbohydrate to BC (S/BC) ratio should be greater than unity (Dinić *et al.*, 2010b). This is because plants do not convert all the WSC to lactic acid during fermentation. Furthermore, the proportion of the S/BC depends on the content of the DM of the ensiled forages (Muck, 1988; Dinić *et al.*, 2010b). If the level of DM is lower, the proportion of WSC to BC (S/BC) has to be higher in order to provide stable pH values (Dinić *et al.*, 2010b).

Wilting the crop sufficiently so that both the growth of detrimental microorganisms is inhibited and the right proportion of carbohydrates and BC is achieved improves the fermentation process (Dinić *et al.*, 2010b). This is because forage DM proportion in the silo is one of the important factors in determining the amount of DM loss and redirecting the fermentation process in the silo mass, especially when the forage is rich in proteins and minerals and low in sugar content (Dinić *et al.*, 2010b). The amount of DM and the forage osmotic pressure in the silage or forages is increased by wilting and the LAB remain active in the environment with high osmotic pressure, while most other anaerobic microorganisms do not survive (Dinić *et al.*, 2010b; Dinić *et al.*, 2005; Muck and Hintz, 2003). Osmotic pressure is the force of attraction of water molecules by the concentrated solutions in relation to the less concentrated solution (Dinić *et al.*, 2010b).

The Table 1-9 below shows the ideal ensiling characteristics for typical forage crops such as maize and grasses compared to lucerne.

Table 1-9: Chemical composition and buffering capacity of lucerne (*Medicago sativa sativa*, L.) and other typical forage crops in g/kg DM

Forage Type	WSC	Crude Protein	WSC/CP. Ratio	Buffering Capacity (meq / kg DM)	Aptitude for Silage making
Maize	80-100	80-100	1.0-3.0	150-300	High
Grasses	35-300	100-160	0.4-1.8	250-550	Intermediate
Lucerne	20-150	140-200	0.1-0.75	350-650	Low

Source: Wattiaux, (1999), WSC= Water soluble carbohydrates, BC=Buffering Capacity, meq=milli equivalent, CP= crude protein

Forages with high BC like lucerne (Table 1-9) are conserved or ensiled by using starter cultures in order to produce high quality feed/silage by ensuring that there is an immediate decrease in pH to prevent growth of undesirable microorganisms, such as clostridia (Dinić *et al.*, 2010b). These starter cultures or inoculants are either chemical additives or biological inoculants.

1.5 The role of forages in dairying

In dairying the three main roles of forages in dairy rations are:

- 1: To maintain rumen health and function through fibre ingestion
- 2: To enhance milk production through both protein and metabolisable energy provision
- 3: To enhance growth and body maintenance through protein provision from legumes and other forage crops (Linn and Kuehn, 1997; Shroeder, 1996; Stokes, 2002).

The maintenance of healthy rumen function and the provision of both protein and energy for milk production are the functions of the forages, which tends to have low digestibility, thereby limiting the animals' nutrient intake (Jung *et al.*, 1996).

The physical coarse forms of forages stimulate rumination and salivation for a health rumen environment, increasing the growth of ruminal microbes, contractions and the passage rate of the digesta through the rumen, as well as increasing the milk fat percentage (Shroeder, 1996). Rumination and salivation increases feed appetite compared to non-forage sources of fibre feed (Bocher, 2010). In dairy cows, as ruminant livestock, forages are essential for the provision of fibre to slow the movement of feed in the alimentary canal, thereby maximising the nutrient absorption process from the consumed feed (Bocher, 2010). The exclusion or insufficiency of forages in dairy cow ration results in both short term metabolic upsets such as low milk production and disease occurrences, and long term upsets, like culling, which result in economic losses to the farmer (Stokes, 2002).

Legume and grass forages with high quality standards are good sources of proteins, energy, fibre and minerals to dairy cows and heifers (Maiga *et al.*, 1997). Legume forages are capable of providing up to three quarters of the protein requirements for lactating dairy cows (Bocher, 2010).

In ruminants, forages provide both ruminal degraded protein (RDP) used in ruminal microbes production and ruminal undegraded protein (RUP) that pass the rumen without being degraded by microbes (Broderick, 1995). In grass and legume forage dairy cows' diet, low protein efficiency in the rumen occurs when there is a rapid and high extent of ruminal protein digestion (Broderick, 1995). Muck (1987) reported that the nitrogen (N) present in forages is in the form of both protein and non-protein nitrogen (NPN). Feeding dairy cows in early lactation requires provision of high protein feeds and, when legume silages are used, other feeds high in RUP should be included (Broderick, 1995).

1.5.1 Forage classification and nutrient composition in dairy cow farming

Forages are classified into two groups; grasses and legumes (Paulson *et al.*, 2008). The nutrient composition of grasses and legumes is influenced by different factors such as: species, stage of maturity, amount of fertilizer applied and soil fertility, environmental conditions of forage growth and time of harvesting (Paulson *et al.*, 2008). Grass forages have higher ADF and NDF concentrations in both leaf and stems than legumes (Paulson *et al.*, 2008). Buxton and Redfearn, (1997) reported that lucerne and red clover plant leaves and stems contain approximately 250 g/kg DM and 400-550 g/kg DM NDF respectively at the same maturity stage of mid flowering, whereas the leaves and stems of tall fescue, smooth brome grass and orchardgrass contain 500 g/kg DM and 700 g/kg DM NDF respectively. Leguminous forages have high nutrient contents with higher feeding values, dry matter intake and animal production than grasses, as well as fixing nitrogen in the soil (Laidlaw and Teuber, 2001).

Forage quality is based on digestibility with reference to the proportion of NDF and ADF and the amount of nutrients it contains (Linn and Kuehn, 1997). Forages are usually the vegetative parts of plants containing more than three tenths NDF with lower concentrations of metabolisable energy and various concentrations of protein and minerals (Wattiaux and Howard, 2011). The fibre content of legume and grass forages is a measure of the

degree of forage quality (Linn and Kuehn, 1997). The quality of forages is based on the forage digestibility proportion and in dairy cows' ration, digestibility rate should be between 680 and 740 g/kg DM ingested, to maintain good passage and utilisation of the ration for the cow to successfully chew the cud and to maintain enough useable fibre in the ration (Shroeder, 1996).

Legume forages' crude protein content ranges from 150-230 g/kg DM with respect to stage of maturity, whereas grasses and crop residues, such as straw, are low in crude protein percentages, ranging from 80-180 g/kg DM with respect to the level of nitrogen fertilization and 30-40 g/kg DM crude protein respectively (Shroeder, 1996).

Fresh legume forages and grasses have more true protein than conserved forages, with 100-150 g/kg DM, and less NPN in the form of peptides, free amino acids and nitrates (Paulson *et al.*, 2008).

Some legumes and grasses contain anti-quality or anti-nutritional factors such as alkaloids, nitrates and prussic acids which result in low animal performances and have adverse impacts on animal health, despite being highly digestible (Rivera and Parish, 2010). Therefore, high quality forages should have high DMI with no adverse effects on animal health and should be able to give economically acceptable animal performances in terms of milk production, reproduction rate and daily average weight gain (Rivera and Parish, 2010).

Forages are usually higher in minerals such as calcium, potassium and other trace minerals than concentrates, lower in phosphorus, and higher in vitamins, especially fat soluble vitamins (A, D, E, and K) than concentrates. However, legume forages have high proportions of vitamin B (Shroeder, 1996). The concentration of minerals in the form of macro- and micro-minerals and ash is higher in legumes than grass forages (Paulson *et al.*, 2008).

The amount of calcium in legumes is about 20- 30% more than that of grasses, whereas phosphorus and potassium concentration is slightly more or equal to that of grasses (Paulson *et al.*, 2008). However, the major factors affecting forage mineral composition are fertilizer application, maturity stage and environmental conditions (McDowell and Valle, 2000; Jukenvicus and Sabiene, 2007).

Ralph *et al.* (1998) reported (in Paulson *et al.*, 2008) that in most grasses, tissue lignification takes place as they advance in maturity as compared to legume forages, which do not undergo tissue lignification, and that hemicellulose are bonded to lignin in grasses through cross linked ferulic acid molecules (Figure 1-2). The high proportion of lignin is directly responsible for the low digestibility of dry matter in grass forages but legumes have higher rates of digestible NDF than grasses (Buxton and Redfearn, 1997). In legume forage leaves, the proportion of cell materials does not increase very much with advances in maturity as compared to grass leaves because a smaller amount of tissue acquires thick secondary walls, whereas in stems of both grasses and legumes, cell wall material proportions and lignification increase greatly with advanced maturity (Jung and Engels, 2002). Åman and Lindgren (1983) stated that as both legume and grass forages flower, the amount of cell wall material increases in grasses, whereas legume forages increase in both leaf and stem proportions. In forages, only xylem is lignified making the cell walls indigestible, whereas in some legumes tissue lignification is absent, rendering them more digestible than grasses (Wilson and Kennedy, 1996). The chemical differences in the lignification of legume and grass cell walls could be the reason for the differences in their digestibility coefficients (Buxton and Russel, 1988). Legume forages have lower rumen fill, a higher digesta passage rate and a higher DMI in ruminants and this could be because of higher digestibility in legumes compared to grass (Dewhurst *et al.*, 1996; Kuoppala *et al.*, 2009).

The digestibility of grass leaves and stems is slightly less than that of legume leaves and stems and in legumes, stems are more susceptible to decreased

digestibility with advances in maturity than leaf blades are (Buxton and Redfearn, 1995). Buxton *et al.* (1995a) stated (in Buxton and Redfearn, 1995) that the decrease in forage digestibility, especially in lucerne and trefoil (*Lotus corniculatus* L.), moving down the stem is at the rate of 20 g/kg per node. In legume forages, there are very few total cell wall materials with higher concentrations of pectin than grass forages when immature, and both cell wall concentration and lignin fractions increase with advanced stages of growth in both legume and grass forages (Paulson *et al.*, 2008). However, legume forages might have higher proportions of cell wall concentrations and lignin when more leaves are lost during cutting (Paulson *et al.*, 2008). Cherney *et al.* (2004) reported that the first cutting of both orchardgrass and fescue showed higher (200-250 g/kg) NDF digestibility than the first cutting of lucerne (480 g/kg) with equal milk production for diets containing orchardgrass, fescue and lucerne. The second cutting of orchardgrass and fescue showed equal NDF digestibility to lucerne. Dairy cows on orchardgrass and fescue produced less milk than those fed on lucerne. Similarly, Weiss and Shocky (1999) reported (in Paulson *et al.*, 2008) that milk yield and dry matter intake between 520 g/kg NDF orchardgrass and 400 g/kg NDF lucerne were the same but the NDF digestibility for orchardgrass and lucerne was 750 g/kg and 490 g/kg respectively.

‘Cross linking of lignin and cell-wall polysaccharides’

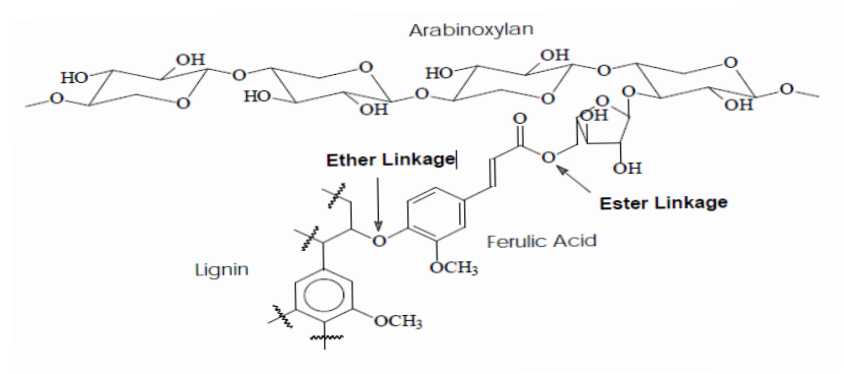


Figure 1-2: "Cross linking of lignin to cell- wall polysaccharides is expected to limit cell-wall digestion"

Source: Jung *et al.* (1996)

1.6 Methane

Methane (CH₄) is one of the greenhouse gases (GHGs), which also include CO₂, Chlorofluorocarbons (CFCs) and N₂O (Moss *et al.*, 2000). Although CO₂ is universally thought to cause greater global warming effects, CH₄, N₂O, and CFCs have great radiative effects, with CH₄ being rated as one of the most important GHGs because its radiative potential is either 25 times or 20 times greater than that of CO₂ (Sejian *et al.*, 2010; Yan *et al.*, 2010). Agriculture generated 49.5Mt of CO₂e in 2009, 8.7% of the UK total and the source of 40% of all methane and 76% of nitrous emissions (Defra 2011).

1.6.1 Methane emission sources

Livestock contributes about eight-tenths of global agricultural emissions (Alluwong *et al.*, 2011) and Steinfield *et al.* (2006) reported that 86Tg (1Tg = 1 million metric tons), representing 37% of these emissions, come from enteric fermentation in the form of CH₄. Sejian *et al.* (2010) reported that the agricultural sector's GHG emission is at about one-fourth of the total universal anthropogenic GHG emissions. Different agricultural practices are responsible for the emission of about 210-250 g/kg, 600 g/kg and 65-800 g/kg of the total anthropogenic CO₂, CH₄ and N₂O (Moss *et al.*, 2000). Yang *et al.* (2003) stated that the production of domestic animals in the world has greatly increased since the 1990s, largely because of better policy management, improved feeding technologies and increased market requirements/demands. In the past livestock have been reported to be responsible for as much as 18% of global emissions (FAO 2006) however these headline statements are misleading as there are large efficiency variations between industries across the world (O'Mara 2011). The world's methane emission sources have been documented as natural, bio-energy/refuse wastes and agricultural sources (Table 1-10).

Table 1-10: Recent estimates of natural and anthropogenic universal methane emission, Tg/yr

Natural		Energy/ refuse		Agriculture	
Wetlands	115	Gas and Oil	50	Rice fields	60
Oceans	15	Coal	40	Livestock (Enteric CH ₄)	80
Termites	20	Charcoal	10	Animal wastes	25
Freshwater	5	Landfills	30	Biomass burning	40
Burning	10	Domestic wastes	25		
	165		155		205

Adapted from IPCC (1992): IPCC 1992 estimated total global CH₄ production / emission into the atmosphere to be 550 Tg/yr. Tg = 1 million metric tons

Despite the fact that ruminant livestock are advantaged in having the capacity to use pregastric fermentation in the digestion of forages, as compared to monogastric animals, the detrimental effects on the environment of forage feeding is large in terms of methane emission (Baker, 1997; Mirzae-Aghsaghali and Maheri-Sis, 2011). Methane is a colourless and odourless gas, 87 % of which is produced in the rumen and 13 % in the large intestines and is emitted by eructation (Murray *et al.*, 1975).

In a ruminant diet with high forage proportions, acetic fermentation occurs, which increases enteric methane production in contrast to propionic fermentation, which occurs from feeding concentrates (Johnson and Johnson, 1995). Johnston *et al.* (1993) stated (in Johnson and Johnson, 1995) that when dairy animals, as ruminants, are fed at maintenance plane of nutrition there is a loss of methane energy in the range of 60-70 J/kJ of forage gross energy. Enteric CH₄ emission from ruminants represents both dietary and animal energy loss (Johnson and Johnson, 1995). Johnson *et al.* (1993) reported (in Johnson and Johnson, 1995) that this energy loss is in the range of 20-120 J/kJ of feed gross energy intake.

1.6.2 Methane emission in developed and developing countries

The amount of enteric methane production or emission in developing countries is different from that of developed countries because of animal species reared, breed, rumen fluid pH (feed factor), acetate to propionate

ratio (feed factor), methanogen population and composition of feed and concentrate level being fed (Sejian *et al.*, 2010). In developed countries, CH₄ production rate per ruminant animal such as cattle, buffalo, sheep and goat is estimated at 8.39, 7.62, and 0.76 (MJ /animal/day) respectively, whereas in developing countries cattle and sheep produce 5.34 MJ/d and 0.76 MJ/day, respectively (Sejian *et al.*, 2010).

1.6.3 Methane production from ruminant animals

The emission or production of enteric methane in ruminant livestock is called methanogenesis. In ruminant livestock, such as cattle, sheep, buffalo and goats, large proportions of dietary fermentation occurs in the rumen, resulting in the production of large amounts of enteric methane per unit of dietary energy ingested (Sejian *et al.*, 2010). In livestock methanogenesis, methane is a by-product of the micro-organisms' digestion of hydrolysed feed carbohydrate proportions such as cellulose and hemicellulose, pectin, and starch in the rumen, and this gas is released by eructation (Bhatta *et al.*, 2007).

Ulyatt and Lassey, (2001) stated that in ruminant methanogenesis, CH₄ production principally occurs by the fermentation of feed in the reticulo-rumen but about 10-20% CH₄ is also produced in the caecum and large intestines. In methanogenesis some physiological factors of the animal associate with the feed and the methanogens (Ulyatt and Lassey, 2001). The association between methanogens and feed causes differences in methane production. However, the methanogens themselves associate with other rumen microbes (bacteria and protozoa) as they compete for the utilisation of hydrogen to produce methane and other enteric fermentation by- products (Ulyatt and Lassey, 2001). Methanogenesis can be controlled by lowering the rumen partial pressure, which can result in low enteric methane emission (Ulyatt and Lassey, 2001).

1.6.4 Methanogenesis

The principal substrates for the production of methane are hydrogen (H_2) and carbon dioxide (CO_2) which are produced from micro-organisms' fermentation of diet. The ruminal microbes involved in enteric methane productions are called methanogens (Boadi *et al.*, 2004).

In the rumen, the feed dry matter is converted to methane by the integrated activities of different microbial species, with methanogens executing the final step (McAllister *et al.*, 1996) as in Figure 1-3. The ruminal primary microbes involved in diet digestion are bacteria, protozoa and fungi, which degrade proteins, starch and plant cell walls into amino acids and sugars (McAllister *et al.*, 1996). These products from the rumen hydrolysis are then converted to volatile fatty acids (VFAs), H_2 and CO_2 gas, through fermentation by both primary and secondary digestive microbes/fermenters (Boadi *et al.*, 2004) (Figure 1-3).

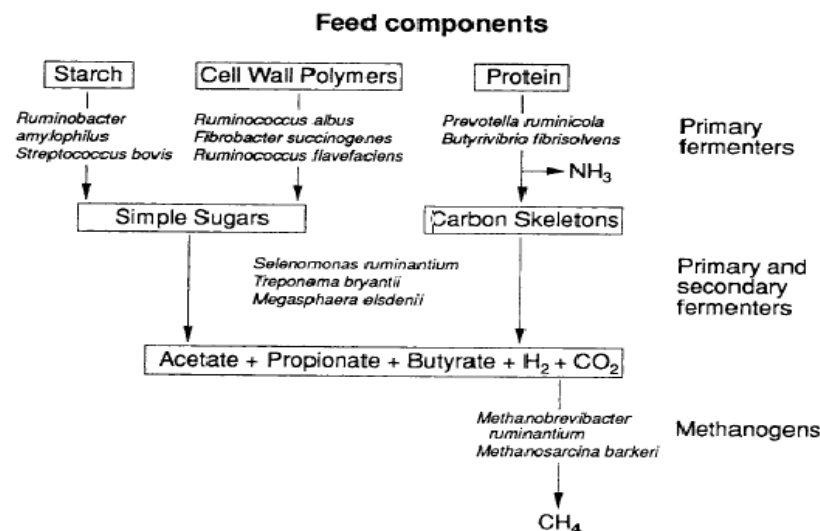


Figure 1-3: Microbial fermentation in the rumen. Primary digestive microorganisms digest feed to simple monomers which are in turn utilised by both primary and secondary fermenters. Methanogens prevent the accumulation of hydrogen by reducing carbon dioxide to methane.

Source: McAllister *et al.* 1996

Methanogenesis is a sequence of chemical reactions occurring in the rumen as represented in the four equations below as described by Boadi *et al.* (2004).



Methane Formation Process. Equation 1; Source: Boadi *et al.* (2004)
Mitigation strategies to reduce enteric methane emission from dairy cows:
Update review

The rumen processes that favour the production of propionate accept protons in the form of H_2 ions, resulting in both low rumen H_2 concentrations and CH_4 production. Conversely, those that favour the production of acetate and butyrate release protons in the form of H_2 ions, increasing both H_2 and rumen methane emission (Hegarty, 1999) as shown in equations 2, 1 and 3.

The amount of VFAs produced in the rumen has an impact on the formation of CH_4 with acetate and butyrate production favouring CH_4 emission, whereas propionate production results in reduced CH_4 emission (Baker, 1997). Increasing the amount of propionate formation in rumen fermentation reduces acetate and butyrate production resulting in low CH_4 emission (Baker, 1997).

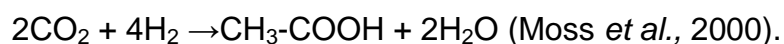
Despite H_2 being the by-product of feed fermentation by the primary microbes (bacteria, protozoa and fungi) in the rumen, it does not build up in it but it is used by methanogens to generate energy for their growth (Boadi *et al.*, 2004). Methanogens reduce H_2 gas pressure in the rumen by using H_2 , while reducing CO_2 to CH_4 (Boadi *et al.*, 2004) and their absence would result in indigestion of the diet (McAllister *et al.*, 1996).

The stoichiometry of acetate (C₂), propionate (C₃) and butyrate (C₄) VFAs can be used to calculate the amount of CH₄ produced from ruminant animals by using the following equation:

$$\text{CH}_4 = 0.45\text{C}_2 - 0.275\text{C}_3 + 0.40\text{C}_4 \text{ (Moss } et al., 2000).$$

1.6.6 Hind gut fermentation in ruminants

Although methanogenesis has been described as a process that occurs in the rumen, if the forage was not digested in the small intestines or the digesta was not fully digested, unfermented organic matter from the rumen passes into the hindgut where it is digested (Moss *et al.*, 2000). In ruminant livestock such as dairy cows, both ground forage feed and diets with high concentrations of maize starch and about 0.10-0.30 units of easily degradable organic matter end up being digested in the hindgut (Moss *et al.*, 2000). The colonic methanogens use H₂ in reduction of CO₂ to methane (Miller and Wollin, 1986) but in other cases non-methanogenic fermentation takes place in the hindgut in which acetate is produced from the reduction of CO₂ by H₂ (Drake, 1994) as illustrated in the equation:



This use of H₂ is beneficial to the animals because the acetate is absorbed into the blood stream to be utilised as a source of carbon and energy, whereas CH₄ is released/emitted out of the animal's body (Moss *et al.*, 2000). Acetogens are the main bacteria that use H₂ in the hindgut to produce acetate and their population in adult ruminants is estimated at 10⁵ml⁻¹. Acetogens develop in lambs after birth and begin to diminish in number as methanogens grow because acetogens are less competitive in H₂ usage than methanogens (Moss *et al.*, 2000).

1.6.7 Enteric methane measurements

In abatement of ruminant livestock enteric methane production, the amount of enteric methane emissions must be quantitatively measured from different

activities being performed by the dairy cows (Johnson and Johnson, 1995). Methane emission is measured by classical standard methods such as respiration chambers or calorimeters such as whole animal chambers, head boxes (ventilated hoods), ventilated flow-through method with a face mask, sulphur hexafluoride (SF₆) tracer gas techniques and meteorological techniques besides methane emission prediction from ingested diet characteristics by using empirical equations (Bhatta *et al.*, 2007). Enteric methane measurements, besides the above mentioned methods, can be achieved by using a laser methane detector (LMD) (Chagunda *et al.*, (2009). The above mentioned methane emission measurement methods are briefly described as follows:

Respiration calorimeters are used to measure the methane concentration balance in the inhaled and exhaled air through gaseous exchange flowing into and outside the open circuit chamber/calorimeter (Johnson and Johnson, 1995). The animal is enclosed in the open circuit respiration chamber for several days with some important inputs like diet, oxygen and carbon dioxide and outputs like excreta, oxygen, carbon dioxide and CH₄ being measured from the chamber (Bhatta *et al.*, 2007) (Figure 1-4). This method requires that the chamber be tightly sealed with slightly negative pressure ensuring that all leaks are inward to control methane loss (Johnson and Johnson, 1995). This method's advantages include the ability to make precise measurements of both ruminal and hindgut CH₄ emissions (Bhatta *et al.*, 2007). However, this method is expensive in terms of chamber construction, the animal is confined in the chamber and has no choice of feed compared to grazing ruminants and experiences loss of nutrients from the feed and reduced diet maximum intake (Bhatta *et al.*, 2007).

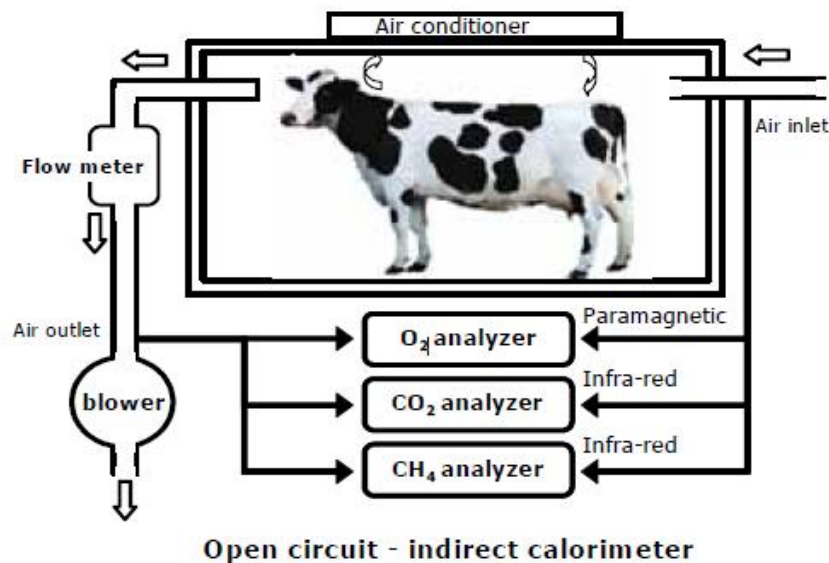


Figure 1-4: Open Circuit- indirect calorimeter

Source: Bhatta *et al.* (2007). Measurement of Methane production from ruminants

Ventilated hoods technique measures CH₄ emissions from ruminants using the calorimetric chamber principles and it involves the use of an airtight box fitted around the head of the cow with either a sleeve or drape around the neck to minimise air leakage (Johnson and Johnson, 1995). The box must have enough room for the animal to move its head flexibly to access feed and water (Bhatta *et al.*, 2007). This method is cheaper than the open circuit calorimeter/ whole animal chamber (Johnson and Johnson, 1995). However, the animal is confined, so this technique requires a restrained and trained animal and also lacks the ability to measure hindgut methane emissions (Bhatta *et al.*, 2007; Johnson and Johnson, 1995).



Figure 1-5: Ventilated hood or headbox. (National institute of Livestock and Grassland Science- Japan)

Source: Bhatta *et al.* (2007). Measurement of methane production from ruminants

Liang *et al.* (1989) stated (in Bhatta *et al.* 2007) that the facemask technique is also used to measure the amount of methane emission from ruminant animals and Johnson and Johnson (1995) stated that this technique uses the same principle as the chamber and hood. This is a simple and lower cost technique and can even be used with grazing animals to estimate CH₄ emissions (Bhatta *et al.*, 2007). Liang *et al.* (1989) reported (in Bhatta *et al.*, 2007) that this technique underestimates heat production and methane emission by 9 % of every measurement.

The facemask method involves the use of a mask for the exhaled air collection which is tightly sealed around the animal's face (Johnson and Johnson, 1995). This causes some stress to the animal and the Douglas bag for gas collection is hard to handle (Bhatta *et al.*, 2007). However, Kawashima *et al.* (2002) came up with a ventilated flow through technique with a facemask comprising of four main parts: main airflow system, air sampling part, gas analyses part and data record and calculation part (Bhatta *et al.*, 2007).

In ruminants, methane emission estimation can be carried out by using Emission from Ruminants Using Calibrated Tracer (ERUCT) techniques with

the tracers being either isotopic or non-isotopic (Bhatta *et al.*, 2007). The isotopic tracer technique uses relatively simple experimental designs with easy calculations for the lower number pools (Johnson and Johnson, 1995) and the methane concentration is calculated from the specific gravity of the radio-labelled methane gas (Bhatta *et al.* (2007). The tracer gas technique uses either (^3H -) methane or (^{14}C -) methane and animals whose rumens have been cannulated (Murray *et al.*, 1975). Methane gas has low solubility resulting in problems in the preparation of isotopic tracer infusions and this serves as one of its limitations for use (Bhatta *et al.*, 2007; Johnson and Johnson, 1995). Non-isotopic tracer methods are also used in methane gas emissions measurements (Johnson and Johnson, 1995). Johnson *et al.* (1994) showed that sulphur hexafluoride (SF_6), an inert non-isotopic tracer gas can be used to measure CH_4 emissions from a herd of animals in a room or during grazing in the pasture field. In an individual animal, methane production measurement is done by infusing a source of SF_6 concentration, which is a permeation tube with a known SF_6 rate of release controlled by a TeflonTM membrane at 39°C , held in place by a porous stainless steel frit and a locking nut, into the rumen before the start of the experiment (Johnson and Johnson, 1995; Bhatta *et al.*, 2007). Each animal under investigation has a halter supporting the inlet tube so that its opening is close to the animal's nose (Johnson and Johnson, 1995). The sampling canister/yoke is vacuumed and as it dissipates, air around the mouth and nose of the cow is sampled or taken and after sampling the canister is pressurised again with nitrogen (Johnson and Johnson, 1995; Bhatta *et al.*, 2007). The sampled gas is instrumentally analysed using gas chromatography (Bhatta *et al.*, 2007).

Johnson *et al.* (1994) in his study with 55 methane emission measurements, using SF_6 technique against 25 chamber measurements of cattle, showed that with 0.90 significance level SF_6 chamber methane estimates, there was no significance difference in the two methods of methane emission measurements. Boadi *et al.*, (2002), in their study with yearling beef heifers, made methane emission comparisons using the SF_6 tracer technique, with mean CH_4 emissions of 137.4 L/day against an open circuit hood calorimeter

with CH₄ emission of 130.0 L/day, in which they found that there was no significant difference between the two methods, with a probability of 0.24.

Tracer gas technique does not confine the animal in one place but allows it to move and even graze in the field, unlike other calorimetric techniques and direct rumen or throat sampling is not required because the use of tracers is responsible for tracer dilution changes from head or air movement (Johnson and Johnson, 1995).

However, SF₆ is a potent greenhouse gas with a global warming effect/potential of 23,900 times that of CO₂ and can exist in the atmosphere for 3,200 years (Bartos, 2002). Bhatta *et al.* (2007) further stated that SF₆ also leaves traces or residues in milk and meat and requires the animals to be trained to wear the halter and the gas collection canister or yoke. This technique furthermore does not measure the hindgut methane, which does not get absorbed into the blood system and is released through the rectum (Johnson and Johnson, 1995).



Figure 1-6: Tracer gas techniques. (National Institute of Livestock and Grassland Science-Japan).

Source: Bhatta *et al.* (2001), Measurement of methane production from ruminants

Meteorological techniques for methane measurement are classified or categorised as either “bottom up” which measures methane production directly from a known number of animals from the ground or “top down” techniques which are land-based methane points established in order to

compare their methane concentration with atmospheric predicted methane/greenhouse gas inventories (Denmead *et al.*, 2000). Only two of these techniques will be briefly described here.

Lockyer and Jarvis (1995) and Lockyer (1997) reported that in this technique air was drawn over animals such as sheep and calves, which were enclosed in a polythene-clad tunnel situated over a grazing pasture measuring 4.3m x 9.9 m. The animals were enclosed for 10 days and the amount of CH₄ production was estimated at an average of 13-14 g/d from sheep and 74.5 g/d from calves. They observed that CH₄ emission decreased with time, which might be associated with the decreasing food supply in the tunnel. The tunnel technique is not a proper method for comparing the differences between two imposed animals in methane emission experiment (Denmead *et al.*, 2000). Harper *et al.* (1999) and Leuning *et al.* (1999) put the animals in a 22 m x22 m enclosure and gas samples were taken from many ports up to a height of 3.5 m of the enclosure in order to evaluate the differences of the mass balance approach. Anemometers were situated at equal height and place/position with the sampling ports to measure the speed of wind (Leuning *et al.*, 1999). The gas samples were measured online using Fourier transmission infrared (FTIR) spectroscopy (Leuning *et al.*, 1999). Seven animals from this experiment were subjected to SF₆ tracer methane emission measurement technique (Leuning *et al.*, 1999). The techniques resulted in similar CH₄ mean values of 11.7 g/d and 11.9 g/d for SF₆ technique and mass balance measurements respectively. This method requires a large number of animals per ha to get a suitable gas concentration for measurements to be taken (Bhatta *et al.*, 2007).

There are many meteorological methods for measuring GHG emission, such as CH₄ gas from the atmosphere and some of them are suitable for CH₄ gas measurement at paddock scale (Denmead *et al.*, 2000; Harper *et al.*, 1999). Judd *et al.* (1999) measured methane emission for five days from a sheep grazed paddock using a micrometeorological flux gradient technique. This involved air samples being drawn from a tower at the height of 3.8 m and 1.2 m on the experimental area downwind boundary with measurement of wind

speed and direction recorded. The amount of methane produced from this technique, which was 19.5 ± 4.8 g/d, compared favourably with that from SF₆ tracer technique, which was 19.4 ± 4.2 g/d (Judd *et al.*, 1999). This technique is not flexible because it requires a dry day with no rainfall, the wind blowing in one direction and can be easily affected by animal movement (Judd *et al.*, 1999). This method cannot measure CH₄ emission differences from two different experiments but within groups of animals (Bhatta *et al.*, 2007).

Laser methane detector (LMD) is a piece of remote methane measuring equipment (Tokyo Gas Engineering, 2006). The concentration of methane between the animal and the equipment is measured through infrared absorption spectroscopy in parts per million-metre (ppm-m) (Tokyo Gas Engineering, 2006). The operating specification for the LMD is for temperatures in the range of 0-40°C, humidity of 20-90% and it conforms to EN61000-6-4:20011 and EN61000-6-2:1999 (Tokyo Gas Engineering, 2006). It was developed to estimate or detect gas emission from gas transmission networks, landfill sites and other methane leaking areas, or where methane build up is a danger (Chagunda *et al.*, 2009). The laser methane detector detects the presence of only methane gas and no other gases such as butane and propylene (Tokyo Gas Engineering, 2006).

The methane measurements are kept in the internal memory of the equipment which is downloaded after completing the measuring process (Tokyo Gas Engineering, 2006). It is recommended that the measurements be done at a 3m distance (Chagunda *et al.*, 2009). Gibbons *et al.* (2009) stated (in Chagunda *et al.*, 2009) that the 3m distance is necessary in order to let the cow concentrate on its normal activities. The measurements should be carried out at the same time of the day and should be repeated over a period of three days (Chagunda *et al.*, 2009).

1.6.8 Enteric methane emission predictions

Mathematical regression models provide the easiest and cheapest means of calculating the production of enteric methane from cattle without using

complicated and expensive measuring equipment (Ellis *et al.*, 2007). Most of these models have successfully predicted enteric methane emission in dairy cows, although their input variables are not commonly chemically analysed and some of the models fail to predict emissions when used outside the range of the input variables they were developed on (Wilkerson *et al.*, 1995). The feed quality characteristics are used in prediction of enteric methane emission (Johnson and Johnson, 1995).

The quality characteristics of dairy cow diet are important in enteric methane emission prediction (Bhatta *et al.*, 2007). Sejian *et al.* (2010) stated that methane predictions usually involve the use of equations with both diet and animal characteristics such as DMI, carbohydrate intake, feed digestibility fractions, feed energy, animal size, milk components and feed digestibility proportions. Enteric CH₄ emission prediction models are categorised as statistical models and dynamic mechanistic models (Kebreab *et al.*, 2006). Statistical models directly use the relationship between the animal's feed nutrient intake and CH₄ emission, and dynamic and mechanistic models simulate ruminal CH₄ emissions by using mathematical explanations based on ruminal fermentation biochemistry (Kebreab *et al.*, 2006; Mills *et al.*, 2003).

Statistical methane prediction regression equations were developed based on calculations from calorimetric experiments (Bhatta *et al.*, 2007). Most enteric methane emission predictions in ruminant livestock are based on Blaxter and Clapperton (1965) equation (Bhatta *et al.*, 2007), which uses DMI and feed digestibility as input variables (Kebreab *et al.*, 2006).

$$\text{CH}_4 (\% \text{GE}) = 1.30 + 0.112 + L (2.37 - 0.50D)$$

Where D= energy digestibility at maintenance level of feeding, L= feeding level.

This regression equation was developed from a number of methane production measurements from mature sheep fed different types of feeds (Johnson and Johnson, 1995).

Moe and Tyrell (1979) developed another statistical regression methane prediction equation based on high quality dairy rations fed to cattle using feed characteristics like soluble residues, hemicelluloses and cellulose as follows:

$$\text{CH}_4 = 3.406 + 0.510(\text{soluble residues}) + 1.736 (\text{hemicelluloses}) + 2.648 (\text{cellulose})$$

Where, CH₄ is in MJ/day and soluble residues, hemicelluloses and cellulose in kg fed/day.

Despite statistical models being fairly successful in predicting methane emissions from diet, many input variables are not measured or chemically analysed, rendering them hard to predict CH₄ emissions outside the mean feed characteristic values they were developed from (Johnson and Johnson, 1995; Wilkerson *et al.*, 1995). The extant methane predicting regression equations such as the one developed by Moe and Tyrell (1979) have limited methane prediction ability because it is hard to find reliable input variables (Sejian *et al.*, 2010). These models give at least a high degree of methane emission prediction but their predictions are not certainly accurate (Benchaar *et al.*, 1998; Johnson and Johnson, 1995). These inefficiencies in methane prediction may be corrected by using regression equations with variables which are mostly measured and formulating models with minimum input variables from many sources (Bhatta *et al.*, 2007).

Many dynamic mechanistic models for dairy cow enteric methane emission estimation have been formulated (Mills *et al.*, 2001; Baldwin *et al.*, 1987). The Mills *et al.* (2001) dynamic mechanistic model predicts enteric methane emission based on simulation of ruminal fermentation from a variety of nutrition conditions. Benchaar *et al.* (1998) reported that for dynamic

mechanistic models measurements to be more accurate than simple statistical equations, a large number of feed nutrient compositions should be used as input variables. Mechanistic and regression equations' methane predictions are possible only when the input variables and feed intake data are available and the rumen functioning conditions are not disturbed (Johnson *et al.*, 2001). A number of available literature on methane prediction states that mechanistic models are more accurate in methane prediction than the statistical models in dairy farms (Sejian *et al.*, 2010).

1.6.9 Enteric methane mitigation strategies

The world nations that ratified the 1992 Framework Convention on Climate Change (FCCC) are responsible for the provision of regular inventories and the introduction of policies and measures to curb GHG emissions for the United Nations (Ulyatt and Lassey, 2001; Moss *et al.*, 2000). Morad (1999) stated (in Mills *et al.*, 2001) that an international action under the Kyoto Protocol was established in 1997 to bring the atmospheric concentration levels of the six GHGs, including methane, to a stable level. Under the 2008 Climate Change Act, the UK is responsible for the reduction of 80% of greenhouse gas emissions by 2050 against a 1990 GHG emission baseline (Lewis, 2008; OPSI, 2008). Scotland has its own Climate Change Act, which aims to reduce GHG emissions by 42 % and 80 % by 2020 and 2050 respectively (Renwick and Wreford, 2011; Sheane *et al.*, 2011). The GHG emissions from milk production/dairy farming in Scotland in 2007 was estimated at 1.5 mtCO₂e and the total GHG emission from milk production, processing and consumption was estimated at 1.7 MTCO₂e, representing 3.0 % of total GHGs emission of Scotland (Sheane *et al.*, 2011).

Howden and Reyenga (1999) reported that global endeavours like the Kyoto Protocol are being carried out to force the reduction of methane emissions or reduce further methane production. However, there is an intention to alter the livestock management systems to decrease CH₄ emission without adversely affecting the production efficiency of the animals and this has been the central idea in research to improve agriculture's environmental

sustainability (Sejian *et al.*, 2010). Many possibilities for decreasing methane production from livestock have been put forward, such as increasing the efficiency of animal production, reducing livestock numbers, exploiting variations between animals, the use of anti-methanogenic feed additives, immunisation, and manipulation of the rumen microbial ecosystems (Ulyatt and Lassey, 2001).

Improving the efficiency of ruminant livestock production characteristics and in particular the milk production of dairy cows will result in a decrease in CH₄ emissions per unit of milk produced (Ulyatt and Lassey, 2001). This can be achieved by genetic improvement of the animals to increase production per unit of feed consumption, and dietary/feed quality improvement, in order to improve DMI in dairy cows by improving feed nutrient composition (Ulyatt and Lassey, 2001).

The type of diet fed to ruminant livestock has an impact on CH₄ emissions (Moss *et al.*, 2000). The ratio of forage to concentrate in the diet influences both rumen fermentation and the acetate to propionate ratio (Lovett *et al.*, 2004). The fraction of concentrate included in the ruminant forage diet is inversely proportional to methane emission (Yan *et al.*, 2000; Moss *et al.*, 2000). Methane production in dairy cows is reduced by increasing the concentrate fraction but is controlled by both the minimum requirements of the physical structure of the diet to prevent acidosis and to balance the energy intake and requirements to avoid overfeeding in dry and late lactating cows (low producing cows, young cows) (Tamminga *et al.*, 2007).

In ruminants' diet, increasing the fraction of concentrate in the form of soluble carbohydrates like grains, reduces rumen pH, detrimentally affecting methanogens' activities and increases digesta passage rate, resulting in reduced methane emission (Mirzae-Aghsaghali and Maheri-Sis, 2011; Moss *et al.*, 2000). The DMI in ruminants increases with greater fractions of concentrates, which will increase the methane emission. However, when it is expressed per unit gross energy intake, a reduction in methane is found and methane reduction is very high when concentrates constitutes the larger

proportion of the diet (Johnson and Johnson, 1995). The increase in the concentrate proportion in dairy cows' forage-based diet increases the ruminal VFA concentration, increasing the proportion of propionates and decreasing the proportion of either acetate or butyrate because concentrates are less structured and this reduces methane emission (Mirzae-Aghsaghali and Maheri-Sis, 2011; Moss *et al.*, 2000). In dairy cows, increasing the concentration of non-structured carbohydrates in the forage-based diet by one quarter reduces methane emission by one fifth, with some adverse effects in ruminant livestock's health such as acidosis, laminitis and fertility problems (Moss *et al.*, 2000).

Methane emissions could be reduced by feeding ruminant livestock on non-fibre carbohydrates, clovers and grasses with high soluble carbohydrates (Lovett *et al.*, 2004). Improving ruminants' forage-based diets by using lower fibre forages with high levels of soluble carbohydrates, (using smaller carbon chain carbohydrates grasses), and grazing on less immature grasses results in lower methane emission (Beuchemin *et al.*, 2007; Ulyatt and Lassey, 2001). A unit amount of ingested and digested cellulose produces three and five times the level of methane gas compared to hemicelluloses and soluble starch or sugars (Moe and Tyrell, 1979). This is because cellulose and hemicelluloses ferment slower than non-structured carbohydrates, resulting in increased methane production per unit of digested cellulose and hemicelluloses (McAllister *et al.*, 1996). The increase in the quality of forage improves the voluntary DMI and decreases the forage rumen retention time, enhancing energetically more good post-rumen digestion and this reduces the fraction of feed/dietary energy converted to methane (Blaxter and Clapperton, 1965). Enteric CH₄ emission in ruminant animals increases with advanced maturity of forages, irrespective of forage type, but the fermentation of legume forages in the rumen produces less CH₄ than grass forage (Moss *et al.*, 2000). Benchaar *et al.* (2001) reported a decrease of 21% in methane emission levels when replacing timothy hay with lucerne in the methane prediction model. Johnson and Johnson (1995) reported that the decrease in methane emission in legume forages, such as lucerne, is

because legumes have lower non-structural carbohydrates and faster rumen passage rate, which favours higher propionate production.

Poor quality forages are responsible for the loss of a high amount of ruminant diet digestible energy, which normally ranges between 150-180 J/kJ of dietary energy in the form of methane (Blaxter and Clapperton, 1965). Increasing the digestibility of feed increases the energy available to the animal/cow and decreases the methane emission per kg production (Allard, 2009). This means that forages with high digestibility have reduced levels of methane production (Allard, 2009).

In dairy cow feeding, methane production increases when mature dry forages are fed or when they are coarsely chopped rather than finely ground or pelleted (Le Liboux and Peyraud, 1999). Fine ground forage diets reduce the molar proportion of acetic acids and increase the molar proportions of propionic and valeric acids, resulting in low methane emission (Le Liboux and Peyraud, 1999). However, fine ground forage diet has proved to be very uneconomical to dairy farmers because of high incidences of acidosis and lowered milk fat percentages due to a lower effective fibre content in the finely ground forages (Boadi *et al.*, 2004, Russell *et al.*, 2007). The process of pelleting and grinding the feed is very uneconomical to the farmer because they increase more costs than the CH₄ mitigation gained (Russell *et al.*, 2007).

Sundstol (1981) reported (in Boadi *et al.*, 2004) that dairy cows emit less enteric methane emissions when fed ensiled forages compared to dried grass. Johnson *et al.* (1996) reported that 200-400 J/kJ methane reduction occurs when the forages are ensiled. This effect is because the digestion of the forages is faster in ensiled forages as a result of the high degree of fermentation that occurs in silage making (Boadi *et al.*, 2004). Benchaar *et al.* (2001) observed a total methane reduction of 33% (Mcal d⁻¹) in the utilisation of lucerne silage compared to lucerne hay in the methane prediction process using a mechanistic model approach. Silage additives like bacterial inoculants and organic acids improve feed quality and palatability

(Boadi *et al.* (2004), which increases DMI (Shingfield *et al.*, 2002) resulting in low enteric methane emissions per unit of dry matter intake (Johnson and Johnson, 1995). This is because ensiling inoculants favours the production of propionic acid to acetic acid during fermentation in the rumen, resulting in low enteric methane production (Russell *et al.*, 2007). The quality of silage, as well as the fraction of silage to concentrate ratio, influences the potential of methane production in any dairy cow ration (Chagunda *et al.*, 2009).

Increasing diet feeding levels in ruminants reduces methane emission as a fraction of the gross energy intake (Boadi *et al.*, 2004). The loss of methane as a fraction of gross energy intake diminishes by 1.6% per multiple increases in intake of the diet (Johnson and Johnson, 1995). This could be because of the high passage rate of feed out of the rumen that reduces feed rumen retention time (Mathison *et al.*, 1998). This reduces the interaction between rumen microbes and organic matter, which in turn decreases the extent and rate of ruminal dietary fermentation, resulting in low methane emission (Mathison *et al.*, 1998; Boadi *et al.*, 2004). Boadi *et al.* (2004) reported that 28% of the differences in CH₄ emission are due to the average retention time and a reduced rumen digesta time that favours the production of propionates which use H₂. The degree at which forage intake levels impacts on the feed passage rate through the rumen is lower compared to concentrates or mixed feed (Mathison *et al.*, 1998).

The digestibility of the feed or diet decreases as the DMI/feeding level increases (Mirzae-Aghsaghali and Maheri-Sis, 2011) and these results in reduced enteric methane emission. The feeding level is the amount of feed consumed per unit weight of the animal (Boadi *et al.*, 2004). Pelchen and Peters (1998) reported that high feeding levels decreases the proportion of gross energy lost as methane. This concurs with the fact that methane emission (g/day) increases but methane yield as a fraction of energy decreases with an increasing feeding level (Mirzae-Aghsaghali and Maheri-Sis, 2011). Methane emissions increase because of higher energy intake but the fraction (%) of gross energy lost in the form of methane produced decreases because very little feed energy is digested at high feeding levels,

resulting in a decrease of energy lost as methane of the total gross energy (Mirzae-Aghsaghali and Maheri – Sis, 2011).

Sutton *et al.* (1986) stated that low diet feeding frequencies favour propionate production, reducing acetic acid production and CH₄ emission in dairy cows. Sutton *et al.* (1986) and Shabi *et al.* (1999) attributed this effect to the high ruminal pH fluctuations (decreasing pH values) and Sutton *et al.* (1986) reported that lower rumen pH results in decreased numbers of methanogens. The daily low meal feeding frequencies increase daily fluctuations in the rumen acidity, which can reduce or prevent methanogens' growth (Russell *et al.*, 2007). This enteric methane mitigation strategy increases losses to the farmers because productivity is adversely affected by low feeding frequency (Russell *et al.*, 2007).

Enteric methane production from legume and legume-grass mixture diets/feed is lower than grass-based diets or grazing animals on legumes fed at the same DMI level due to condensed tannins and saponins found in some legumes (Dewhurst *et al.*, 2009).

Saponins are natural detergents occurring in many forages and they contain surfactant or detergent characteristics. This is because of the presence of water-soluble and fat-soluble components, such as a fat-soluble nucleus with steroid or triterpenoid structure and one or more WSC side chains (Mirzae-Aghsaghali and Maheri-Sis, 2011). It is reported that condensed tannins are either directly antimethanogenic or indirectly inhibit hydrogen production in the rumen because of decreased diet digestion (Mirzae-Aghsaghali and Maheri-Sis, 2011). Pen, (2007) reported that the main commercial saponins are *Yucca schidigera* and *Quillija saponira* found in the arid Mexican desert and arid areas of Chile respectively. In sheep fed on forage containing saponins, the number of protozoa in the rumen decreases and this promotes the flow of microbial proteins from the rumen, increasing feed utilization efficiency and reduced methanogenesis (Calsamiglia *et al.*, 2007 ; Pen, 2007).

Forage/plant secondary compounds, such as tannins and saponins, have been tried in CH₄ mitigation strategies because they have the natural capacity to oppose chemical additives (Mirzae-Aghsaghali and Maheri-Sis, 2011). Forages which contain tannins have an anti-methanogenic activity because of the condensed tannins (Mirzae-Aghsaghali and Maheri-Sis, 2011). Condensed tannins affect either the ruminal methanogens or the ruminal hydrogen formation because of a reduction in feed degradation or digestion resulting in methane emission (Mirzae-Aghsaghali and Maheri-Sis, 2011). Condensed tannins are flavonoids which combine with soluble proteins in the rumen and the proteins are released in the small intestines, which is an acidic environment (Mirzae-Aghsaghali and Maheri-Sis, 2011). The release of proteins in the small intestines increases amino acid absorption as well as reducing bloat and methane emissions (Mirzae-Aghsaghali and Maheri-Sis, 2011). Legumes, which have tannins such as *Lotuses*, reduce methane emission per unit DMI (Beauchemin *et al.*, 2007) and in ruminants the reduction ranges between 120-150 J/kJ of dietary energy intake (Rowlison *et al.*, 2008). Condensed tannins, being directly toxic on methanogens, reduce methane production by 100-224 J/kJ of dietary energy intake (Grainger *et al.*, 2009). However, increased proportions of condensed tannins in dairy cows' feed reduces rumen feed degradation with consequential low animal productivity (Beauchemin *et al.*, 2007).

1.7 Research Work

This project aimed to include lucerne forage in dairy cows' total mixed ration (TMR) to achieve maximum milk production without any detrimental effect on the dairy cow's characteristics and environment. The specific objectives of this study were to :

- (1) Evaluate the growth rate, effect of weed infestation on yield and the effects of simulated winter leaf defoliation on early spring lucerne growth rate and yield.

- (2) Examine the ensiling of lucerne using biological / microbiological inoculant.
- (3) Formulate diets containing lucerne and estimate methane emission using published equations.
- (4) Investigate the relationship between the laser methane detector enteric methane measurements and the estimated enteric methane emissions

2. Chapter Two: Experiment 1. Evaluation of the effect of weed infestation on lucerne yield in summer and winter season and the effect of winter harvesting on lucerne growth rate and yield in spring

2.1 Introduction

Lucerne (*Medicago sativa, sativa*) is a frost resistant forage crop. In winter it becomes dormant and regrows in spring and summer using the nutrients which were reserved in the plant during favourable growing weather (McDonald *et al.*, 2003; Zanin, 1998; Volenec *et al.*, 1996). It grows well from spring to autumn and grows fastest from spring to early summer (Frame, 1998). However, it does not have a long season for growth compared to crops which do not go dormant in winter (Frame, 1998).

Lucerne (*Medicago sativa sativa*) has high yielding potential per hectare compared to other forage legume crops and is mostly grown without inorganic fertilization (Lancefield *et al.*, 2009; Frame, 1998). Lucerne has a harvesting cycle of 40 days depending on weather and produces about 10-12 t/ha DM herbage annually (Limagrains, 2011; Zanin, 1998). Although lucerne has a high yielding potential and nutritive value, it does not compete favourably for nutrients and sunlight with other plants, such as docks, resulting in reduced growth rate and yield (Frame, 1998).

It is well documented that frequent harvesting of lucerne adversely affects shoot growth and stand persistence because of depletion of carbon and nitrogen reserves in roots required for regrowth (Avice *et al.*, 1996; Dhont *et al.*, 2002). Lucerne accumulates sugars and nitrogen in autumn in the roots for frost tolerance in winter and spring growth (Dhont *et al.*, 2002). It is expected, therefore, that the harvesting of lucerne in winter may affect its spring growth rate and yield.

This study aimed to evaluate the growth rate of lucerne in a field which also contained other plant species (referred to as weeds) in both summer and

winter season and the effect of winter harvesting on the lucerne growth rate and yield at the first spring harvesting.

2.2 Materials and Methods

Lucerne was grown at SAC Dairy Research Centre at Crichton Royal Farm, Dumfries, UK, situated at longitude 3.37 and latitude 55.04 N in the South West of Scotland. Lucerne was sown in summer on 27th May 2010 on a 2.5 ha land. The land on which grass was previously grown was sprayed, ploughed and lime was applied at the rate of 5 tonnes per hectare before seedbed preparation to raise the soil pH from 6.7 to 7.0. The land had fine silt mixed with clay soil. Approximately 35 m³ of organic fertilizer in the form of cattle slurry was applied before broadcasting the seeds, because lucerne uses high amount of nutrients during growth. The variety of lucerne that was grown was Marshal and the seeds were broadcast at the rate of 30.9 kg/hectare.

Lucerne growth rate in summer was assessed at one week intervals for a period of seven weeks. Lucerne and weeds were harvested from 15 April to 29 May 2011. Three points were randomly selected at each sampling day by throwing a coloured marker. Points where the crop had been previously harvested were marked and not used if the marker landed at these points.

The total biomass of lucerne and weeds were harvested from a 1 m² area, which was randomly selected, using a 1 m square quadrat by hand-cutting with a hedge shear to ground level. The total harvested fresh biomass was botanically analysed by separating lucerne from weeds. The lucerne forages were further separated into leaves and stems and were weighed to find the fresh weight basis. The whole harvested fresh lucerne leaves and stems were then dried for 24 hours in the laboratory oven at 100° C to find their dry matter yield. Similarly, the whole harvested fresh weeds were weighed to find the fresh weight and were dried for 24 hours in the laboratory oven at

100°C for DM content analyses. After 24 hours drying, the samples were then weighed again and these weights represented lucerne and weed harvest on DM basis.

The growth of lucerne in winter was assessed at two week intervals from 12 January to 22 March 2012 for a period of twelve weeks. Eighteen sampling areas were randomly selected and covered with wire cages of uniform cross-sectional area of about 1.5 x 1.0 m with about 1.0 m height, preventing them from being grazed by migratory birds such as geese. Lucerne and weeds were harvested with a hedge shear by hand-cutting to ground level from the three randomly selected areas covered by the wire cages every two weeks. Each area was only harvested once during the harvesting period and the wire cages were not removed because the sampled areas were used to evaluate the effect of lucerne winter harvesting in spring growth rate and yield. The harvested lucerne and weeds were manually separated or sorted out and weighed to find the fresh weight. A sub sample of the harvested fresh lucerne and weeds were then dried for 24 hours in the laboratory oven at 100° C to evaluate the DM yield. The dried samples were then reweighed and these weights represented the lucerne and weed harvest on DM basis.

The effect of the date of winter harvesting (to simulate grazing by cattle or geese) on lucerne growth rate in spring was assessed from 02 May to 06 June, 2012, by measuring the height of the lucerne forages which was harvested in winter at one week interval for a period of six weeks, using a sward stick. The height of 20 lucerne regrowth plants, from the three plots/points of the eighteen sampling points of 1.5 x 1.0 m cross-sectional area, were measured using a sward stick each week.

The harvested lucerne and weed yields in summer and winter were analysed using one way Analyses Of Variance (ANOVA) in Minitab 15 software to evaluate the difference in the amount of lucerne, lucerne leaves, stems and weeds harvested in summer and winter seasons. Excel 2007 version was used to determine the spring growth trend of lucerne after being harvested in winter season.

2.3 Results

The growth of lucerne was observed from April to May 2011. The highest mean yield of lucerne harvested after seven weeks of growth in summer 2011 was $6.81 \pm (SE=0.36)$ t/ha on dry matter basis with the yield ranging from $1.09 \pm (SE=0.23)$ to $6.81 \pm (SE=0.36)$ t/ha, respectively. The highest mean yield of weeds harvested together with lucerne crop after seven weeks of growing was $1.35 \pm (SE=0.23)$ t/ha DM with the yield ranging from $0.42 \pm (SE=0.07)$ to $1.35 \pm (SE=0.23)$ t/ha.

The lucerne yield harvested indicated that lucerne grows well from April to late May. There was a steady increase in the amount of lucerne yield harvested from April to late May Figure 2-1a and 2-1b. The total lucerne yield was on average constantly higher ($P<0.05$) than weeds yield. The total amount of lucerne leaves harvested at one week intervals was consistently higher than lucerne stems up to 4th May 2011, when lucerne stem yields started increasing and lucerne leaf yield was decreasing. In this study there was a higher proportion of lucerne leaves as a percentage of total lucerne yield (almost 60.6, 57.1 and 50.1 %) harvested at the beginning of the summer season than stems. The harvested yield for both lucerne leaves and stems dropped from $1.83 \pm (SE=0.11)$ and $1.82 \pm (SE=0.04)$ t/ha in the late month of April to $1.30 \pm (SE=0.25)$ and $1.67 \pm (SE=0.31)$ t/ha in the early month of May, respectively. However, the growth of lucerne leaves was decreasing ($P<0.05$) whereas lucerne stems were steadily increasing ($P<0.05$) from mid May 2011 up to the end of the month of May.

However, from early May, the stem DM yield started increasing as the leaf DM was decreasing, whereas lucerne leaves and stems on dry matter basis as a proportion of the fresh basis, the increasing and decreasing of lucerne leaves and stems was apparent towards the end of the month of May (Figure 2-1b). The harvested yield of leaves, stems and total lucerne and weeds were plotted on linear graphs in both t/ha DM and as the proportions of the total fresh yields of lucerne leaves, stems, total lucerne and weeds to

evaluate the trend of the yield harvested in summer as presented in Figure 2-1a, 2-1b and 2-1c.

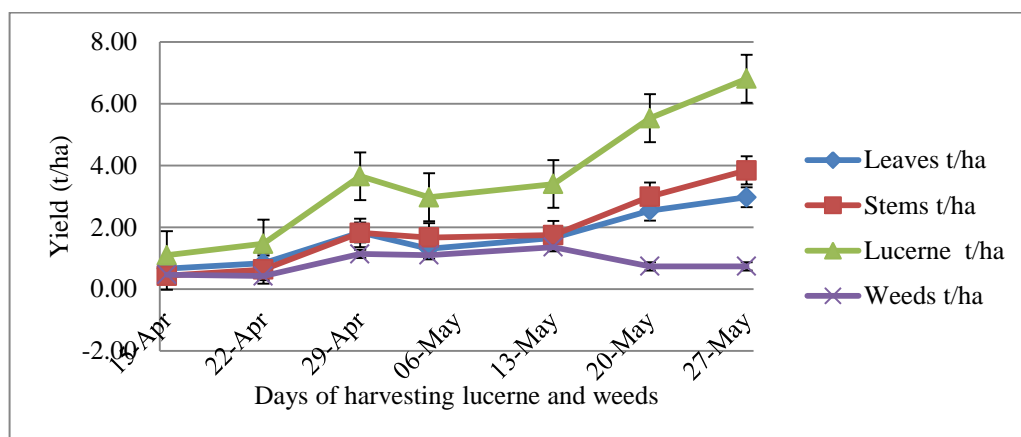


Figure 2-1a: Lucerne and weed yield harvested in 7 weeks of growth in summer 2011. The error bars are standard errors

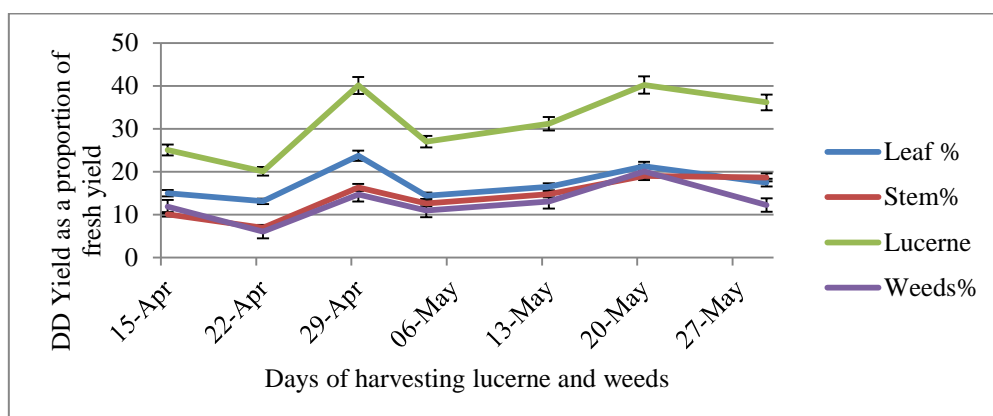


Figure 2-1b: Lucerne and weed DM yields as a proportion of their fresh yield harvested in 7 weeks of growing in summer 2011. The error bars are standard errors

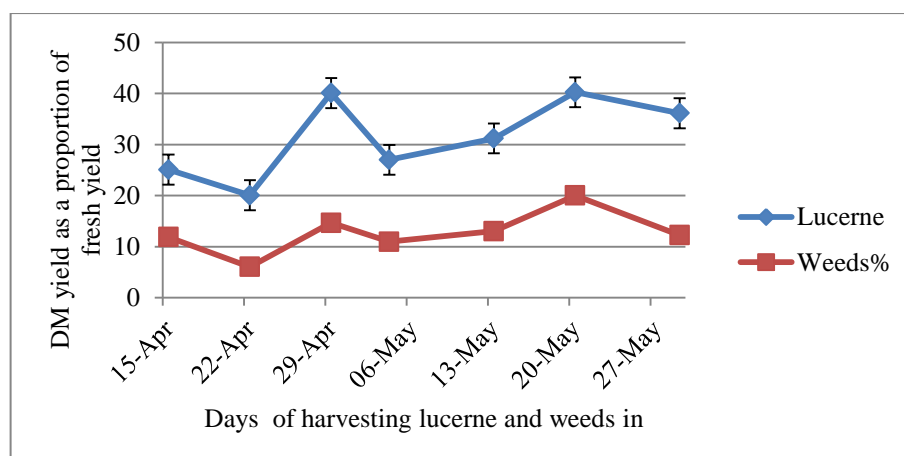


Figure 2-1c: Lucerne and weed DM yield as a proportion of their fresh yield harvested in 7 weeks of growth in summer 2011. The error bars are standard errors

In winter the growth of both lucerne and weeds was monitored and harvesting was done at fortnightly interval because in winter lucerne becomes dormant. The yield results for both lucerne and weeds on DM basis are presented in Table 2-1.

Table 2-1: Lucerne and weed yield harvested in winter 2012

Harvesting date	12-Jan.	25-Jan.	09-Feb.	24-Feb.	08-March	22-March
Lucerne (g/ m ²) Mean(SEM)	25.8(1.21)	25.3(0.58)	24.1(0.10)	31.0(1.09)	20.2(0.66)	19.7(0.26)
Weeds (g /m ²) Mean (SEM)	26.4(1.13)	26.6(1.59)	24.8(1.44)	28.7(3.09)	21.2(0.31)	22.0(1.53)

SEM= standard error mean,

One-way ANOVA: Lucerne and Weeds

Source	DF	SS	MS	F	P
Factor 1	0.9	0.9	0.07	0.799	=0.05
Error 10	10	124.9	12.5		
Total 11	11	125.8			

The mean highest lucerne yield harvested in winter after 12 weeks of growth was $31.0 \pm (\text{SE}=1.09) \text{ g/m}^2$, with the yield ranging from $19.7 \pm (\text{SE}=0.26)$ to $31.0 \pm (\text{SE}=1.09) \text{ g/m}^2$. The mean highest yield of weeds harvested after 12 weeks of growing was $28.7 \pm (\text{SE}=3.09) \text{ g/m}^2$ with the yield ranging from $21.2 \pm (\text{SE}= 0.31)$ to $28.7 \pm (\text{SE}= 3.09) \text{ g/m}^2$. The highest mean harvested yield for both lucerne and weeds harvested at the end of the sampling period, therefore, in tonnes per hectare, was 0.31 t/ha and 0.29 t/ha respectively. The lucerne yield harvested at every two weeks in winter was consistently lower than the weed yield except on 24th February whose lucerne yield was higher by 8.0 % than weeds.

The growth of both lucerne and weeds represented as yields in winter was not vigorous compared to summer yield with reference to the amount of yield harvested at every sampling day (Figure 2-1). In summer, both lucerne and weeds yields were high as evidenced by the sharp changes in yield increase and decrease (Figure 2-1a-c).

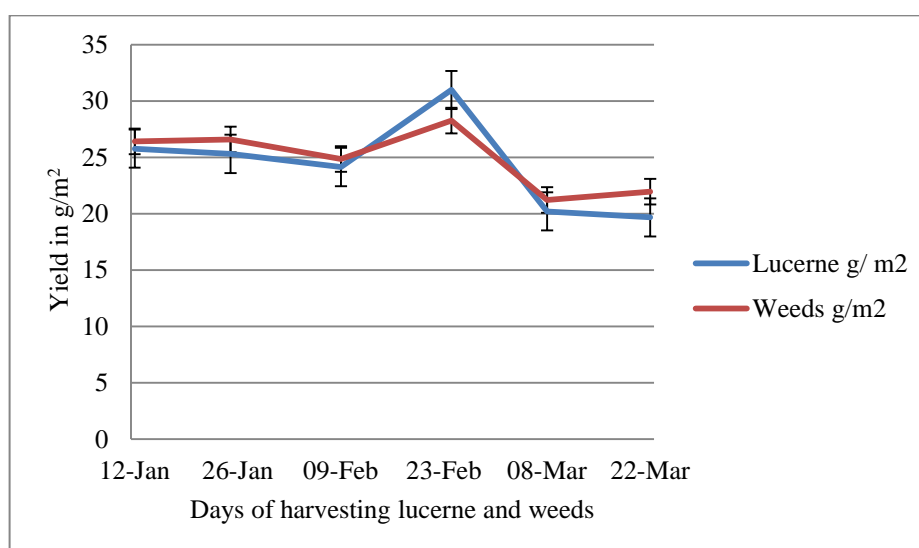


Figure 2-2: Lucerne and weeds yields harvested at two weeks intervals in winter 2012. The error bars are standard error

The summer 2012 growth is shown in Figure 2-3. Lucerne harvested at the onset of winter (12 January and 25 January) grew taller, with a height proportion of 17.4% and 17.6% compared to the height of the lucerne harvested in late winter (22 March). The height regrowth of the lucerne which was harvested in mid-winter (08 and 24 February) was less than those harvested in early winter (12 January) by 1.5% and 8.2% and taller than those harvested in late winter by 15.5 % and 7.8 % .

Although the lucerne regrowth height from the plots harvested on 12 January 2012 was the greatest of all the other plots, the growth rate was constant with very little growth increase changes (Figure 2-3). The lucerne growth height from the fields which were harvested on 08 March and 22 March had more growth increase changes as compared to the growth increase changes of the other fields/plots (Figure 2-3). Figure 2-3 shows that after six weeks of growing, the height of lucerne defoliated on 08 March was equal to the height of lucerne harvested on 24 February. The lucerne fields/plots harvested on 08 March and 24 February grew higher than the lucerne which was harvested on 22 March after growing for 6 weeks by 7.8 % and 7.5 %, respectively (Figure 2-3).

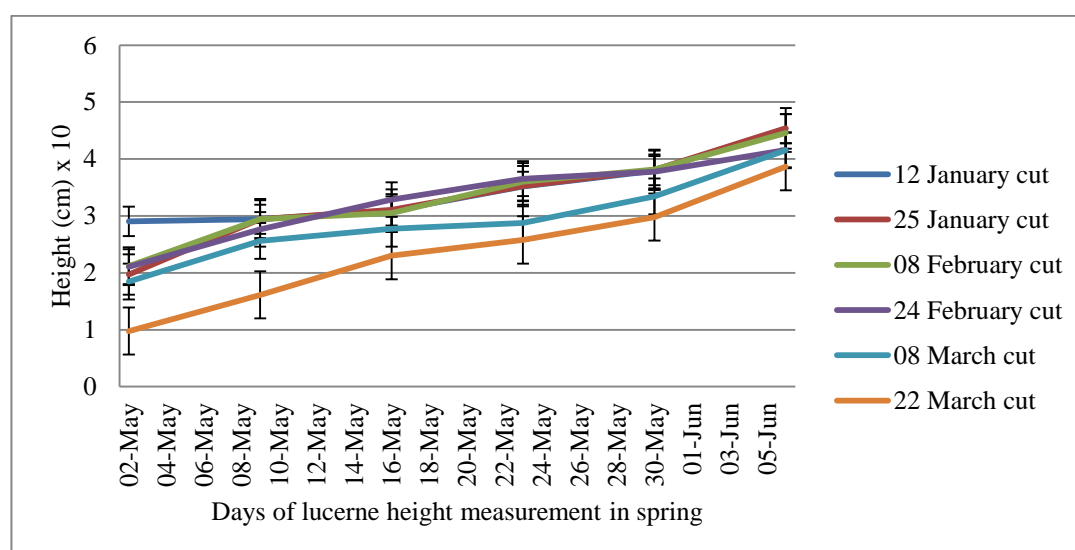


Figure 2-3: The growth of lucerne in spring after winter harvesting. The error bars are standard errors

2.4 Discussion

2.4.1 The yield of lucerne in summer

The lucerne yield harvested indicated that lucerne grows well from April to late May. In this study there was a higher percentage of lucerne leaf (almost 50.1-60.6%) yield harvested than stem from 15th April to 29th May 2011. These results agree with Kokate (1990) who stated that almost 480 g/kg DM of the lucerne plants consists of leaves. Arinze *et al.* (2003) stated that almost half of the lucerne plant mass is composed of leaves. In agreement with Arinze *et al.* (2003), almost equal amount of lucerne leaves and total lucerne biomass as plant was harvested. Similarly, lucerne yield harvested was higher than weed yield. This lucerne growth characteristic, in this study, reflects well with Radović *et al.* (2009) report that lucerne has the ability to produce high yields. This means that in summer lucerne grows faster than weeds, showing that it can easily compete with weeds for nutrients and sunlight.

The total lucerne yield harvested on each harvesting date from April to May 2011 steadily increased, whereas the weed yield was consistently below lucerne yield, with the highest yield harvested within seven weeks of growth amounting to $6.81 \pm (SE=0.36)$ t/ha. The total amount of weeds harvested will depend on level of weed infestation and will vary from field to field. Sheldrick *et al.*, (1995) reported that lucerne yield in the UK, after stand establishment, can be in excess of 10.5 t/ha DM annually. The high amount of lucerne harvested in this study means that lucerne could compete with weeds for nutrients and sunlight, in summer, as opposed to the findings of Frame, (1998) and Phipps *et al.* (1997).

The increase in the amount of stem DM proportions, as compared to leaves, towards the end of May 2011 shows that the lucerne is maturing. This lucerne growth trend increases yield compared to weed yield but McDonald *et al.* (2003) stated that lucerne harvesting at a later stage than the recommended stage of growth increases yields but reduces the quality of the forage harvested. This shows that lucerne has reached a maturity stage

where it is now supposed to be harvested. At this stage of growth, the nutrient content in lucerne, such as protein, will start decreasing while the fibre content, such as NDF, ADF and acid detergent lignin (ADL), will be increasing with a consequential decrease in the forage quality. (McDonald *et al.* 2003)

2.4.2 The yield of lucerne in winter

The yield of lucerne in winter 2012 was on average constant, with the highest lucerne yield harvested being 0.31t/ha compared to 0.29 t/ha for weeds. Lucerne cutting for hay and silage in the UK is usually done 3-6 times annually. In this study, more weeds than lucerne were constantly being harvested every fortnight in winter. This reflects well with the fact that lucerne becomes dormant in winter season (McDonald *et al.*, 2003; Frame, 1998) and the weeds were advantaged in using more nutrients for growth, hence almost equal amount of lucerne and weed yields being harvested.

2.4.4 The effect of the date of harvesting on the winter regrowth height of lucerne

The lucerne taproots are the main nutrient reservoirs for plant nutrients, such as carbohydrates and nitrogen, to support the spring shoot production and regrowth of shoots after being defoliated / harvested (Volenc *et al.*, 1996; Dhont *et al.*, 2002). In autumn, lucerne plants store carbohydrates and nitrogen in taproot reservoirs for use during winter (Volenc *et al.*, 1996; Dhont *et al.*, 2002). This explains the low lucerne early spring regrowth heights for the lucerne which was harvested in the late winter (08 March and 22 March 2012) compared to the height of the lucerne harvested in the early winter period, such as 12 January 2012. In this study, the harvesting of lucerne in early winter has proven to have higher spring regrowth height than late winter harvesting. This is because late harvesting occurred when the lucerne had already used a high proportion of its nutrient reservoirs for its survival in winter. However, a study on the depletion of nutrient reserves in the taproots was not undertaken. This study has proven that harvesting of

lucerne in late winter could adversely affect the regrowth behaviour and final yield of lucerne in spring.

2.5 Conclusion

The findings of this study suggested that the growth of lucerne in summer could not be severely affected by poor weeding management as a result lucerne could be grown in mixed stands with other forage crops. The study has further proven that satisfactory yields of lucerne are possible in South West Scotland. For winter grazing and harvesting management, the study suggested that lucerne could either be grazed or harvested at the onset and before mid-winter by either dairy cows or any other migratory birds without the regrowth and yield in spring being adversely affected. However, late winter grazing or harvesting will significantly reduce spring lucerne growth rate and yield.

3. Chapter Three: Experiment 2 Evaluation of the efficacy of *Lactobacillus plantarum* MTD-1 inoculant/additive on lucerne silage nutrient composition and aerobic stability

3.1 Introduction

The ensiling process of forages is aimed at conserving the biomass and fermentation by-products, together with their nutrient content (Dinić *et al.*, 2010b). However, when forage crops such as lucerne are harvested and stored there is a loss of dry matter content and nutritional value of the feed, resulting in low feeding value (Wattiaux, 1999). During ensiling of forages, hemicellulose is degraded by plant enzymes and acid hydrolyses (Dewar *et al.*, 1963; Morrison, 1979) and this hemicellulose degradation results in silages with lower carbohydrate content than the harvested forages (Chestnut *et al.*, 1988).

Although lucerne has both high yielding ability and nutrient content for dairy production, it presents difficulties in silage making, resulting in low quality silage with less aerobic stability during feedout (Phipps *et al.*, 1997; Muck, 1988; Wattiaux, 1999). There has been very little research in the UK on the use of inoculants when ensiling lucerne at dry matter lower than 300 g/kg.

During ensiling of lucerne as forage crops, proteins undergo proteolysis process resulting in soluble non-protein nitrogen (NPN) such as peptides, amino acids, amines and NH₃ (Muck, 1996; Barnhart and Nadeau, 2008; Kung, 2010, Muck, 1988). McDonald *et al.*, (1991) reported (in Marshall *et al.*, 1993) that lucerne crops which have high protein content, low WSCs and high buffering capacity undergo partial fermentation resulting in high proteolysis. The high buffering capacity of lucerne results in a slow pH decline during ensiling, which encourages the growth of harmful microbes like clostridia bacteria, which degrade proteins (Muck, 1988). The by-products of ensiling, such as lactic acid, acetic acid, propionic acid, butyric acid, ethanol and ammonia-N, are crucial in determining the quality of the silage after ensiling and during silage feedout (Kung, 2010). Excessive

production of these ensiling by-products (except lactic acid) indicates poor fermentation (Kung, 2010).

Additives are usually used in silage making to reduce silage nutrient losses resulting in improved silage quality (Fernandez and O'Kiely, 2008). Silage biological inoculants which contain lactic acid bacteria (LAB), such as *Lactobacillus plantarum*, *Enterococcus faecium* and *Pediococcus species*, are applied before the forages are ensiled to improve the ensiling process (Hu *et al.*, 2009). LAB are effective in reducing the final pH of silages, resulting in low proteolysis and loss of nutrients (Driehuis *et al.*, 1999) but they do not improve aerobic stability of the silages during feedout (Jatkauskas and Vrotniankiene, 2009; Muck and Hintz, 2003). Many biological inoculants have been shown to improve silage nutrient quality (Kung *et al.*, 1984) and improved performance in animal growth and milk production has been observed (Kung *et al.*, 1987). However, very little information is available on biological inoculants which improve silage aerobic stability (Lindgren *et al.*, 1990).

There has been work to evaluate the use of *Lactobacillus plantarum* MTD-1 on lucerne but this work has been with high dry matter forage. The objective of this study, therefore, was to evaluate the efficacy of ensiling lucerne forages at dry matter content less than 300 g/kg, with a microbiological inoculant *Lactobacillus plantarum* MTD-1 on lucerne silage nutrient quality, fermentation characteristics and aerobic stability during feedout.

3.2 Materials and Methods

3.2.1 Site of study and silo preparation

The study was carried out at the SAC Dairy Research Centre at Crichton Royal farm, in Dumfries, UK situated at longitude 3.37 W and latitude 55.04 N in the South West of Scotland. It used materials from the harvest of a third-cut lucerne (*Medicago sativa sativa*), which was made into large bales (approximately 500kg fresh weight) by using an additive free bailer which was previously cleaned. Lucerne was harvested in the morning on 3 August

2010 by using a disc forage mower before being wilted for 48 hours. Two of the bales of fresh lucerne with no additive were chosen, at random, for the experiment, and experimental laboratory silos were filled within 5 hours of the lucerne being baled. The experimental silos were 5 litre screw capped plastic bottles. The forage was chopped using the farm feeder wagon (Strautman) in order to achieve the required chop length of between 50 and 70 mm. The chop length was selected to ensure good compaction within the experimental silos. One bale was chopped through the feeder wagon, to clean the feeder wagon of other feed materials, which were discarded. The second bale was chopped in the feeder wagon for 20 minutes and this chopped lucerne was used to fill the experimental silos.

3.2.2 Treatments

There were two treatments:

(1) Control (C) without additive

(2) Treatment (T) ensiled with Ecosyl 100 *Lactobacillus plantarum* MTD-1 inoculant at the appropriate rate of dosage, which was 1×10^6 colony forming units (cfu) (organisms) g^{-1} of the lucerne forage as per manufacturer's instructions. The experimental additive was sprayed on the chopped lucerne forage and manually mixed together with lucerne before filling the experimental silos.

Fourteen silos of both control (C) and Treatment (T) silos were filled (seven for each treatment) with 500g of the chopped lucerne forages and the forages were pressed with a hand presser to make sure that enough air was removed from the silos (Figure 3-1). The control treatments were completed before the additive treatments to ensure no additive in the control treatments. This technique of ensiling lucerne in plastic silos was the first one to be conducted at Scottish Agriculture College, Dairy Research Centre, in Dumfries.



Figure 3-1: Lucerne forage being packed into 5 L bottle silos using a hand presser

3.2.3 Silo Preparations

Bench top silos, 5-litre (L) screw top plastic containers made by a company called Plysu Containers in Northumberland in England, were filled with the forage, simulating anaerobic ensiling conditions and were labelled with date of filling, whether ensiled either without additives C or treated with additives (T) (LP MTD-1), and the date when the silos would be opened (day 1, day 3, day 7 and day 90 after ensiling). Seven 5 litre silos and 3 smaller 250ml pots were filled for each treatment. The 250 ml silos for each treatment were opened on days 1, 3 and 7, and three 5 L silos from each treatment were opened on day 3, after ensiling to monitor pH changes in each treatment. The remaining 5 litre silos were kept in the same building at an ambient temperature of 5.5° C for 90 days.

3.2.4 Chemical analyses and silage aerobic stability determination

Three fresh lucerne samples for each treatment Control (C) and Treatment (T) (LP MTD-1) on day 0 were sampled before packing them into the silos and stored at ambient temperature for chemical analyses on DM, pH, crude protein (CP), ether extract (EE), water soluble carbohydrates (WSC), Metabolisable energy (ME), neutral detergent fibre (NDF) and ash. The samples were stored in sampling plastic bags and placed in a deep freezer

within 15 minutes and were stored there for 24 hours before sending them for chemical analysis.

The three smaller 250 ml polypots silages of both each control (C) and treatment (T) (LP MTD-1) were opened on days 1, 3 and 7 after ensiling to analyse for pH changes in order to ascertain the degree of fermentation that has occurred. However, as a back-up, three of the seven 5 litre plastic silos for each treatment C and T (LP MTD-1) were also opened on day 3 for pH analyses. The opened silos were then discarded.

Four silos of each treatment Control (C) and treated (T) (LP MTD-1) were opened on day 90, for chemical analyses. The following chemical analyses were done at SAC Analytical Services in Edinburgh: dry matter (DM), crude protein (CP), ether extract (EE), water soluble carbohydrates (WSC), metabolisable energy (ME), neutral detergent fibre (NDF), ammonia-nitrogen and ash. The silos were impermeable plastics with screw top lids; therefore very few DM losses were expected. In the chemical analyses of the silage nutrient chemical composition wet chemistry methods were used. The detailed chemical methods used in the analyses of the samples are presented in Appendix 1.

One sample from each of the four day 90 silages, from both control and treatment, were sent to an external laboratory services for the analysis of lactic acid, ethanol, acetic acid, propionic acid and butyric acid through SAC Analytical Services in Edinburgh.

The ensiled forage samples (four for each treatment) left in the day 90 silos, after sampling for silage nutrient content and fermentation characteristics (volatile fatty acids) analysis, were used to measure the aerobic stability by using Easylog temperature data loggers (Lascar Electronics Limited) whose thermocouple wires were inserted into the centre of the well fluffed/mixed silages. Silage aerobic stability was measured from the remaining 8 silos, four for each treatment, after 90 days of fermentation. The remaining silages in the silos were well fluffed/mixed while they were still in the silos to permit

air circulation. The silos were covered with polystyrene chips for ambient temperature insulation so that the recorded temperatures represented silage temperature changes. The temperature was logged every hour for eight consecutive days measuring changes in temperatures when the silos were exposed to air. The silages were exposed to air for 8 days while measuring their temperature rises and one temperature data logger was used to measure the room temperature. The aerobic stability was measured, by the standard method, as the length of time taken for the silage's temperature to reach 3° C above ambient/ room temperature.

3.2.5 Statistical analyses of the silage chemical nutrient content results

The chemical characteristics of the lucerne silages were analysed by using one-way Analyses Of Variance (ANOVA) and Excel 2010. The analyses of variance were used to find the difference in nutrient content, fermentation characteristics and aerobic stability between *Lactobacillus plantarum* MTD-1 treated and control silages. The Excel 2010 version was used to evaluate the trend in the temperature rises of the silages during the 8 days of measuring aerobic stability.

3.3 Results

3.3.1 The effects of inoculation with *Lactobacillus plantarum* MTD-1 on the silage nutrient composition

The change in pH of the lucerne forages, from both control and treatment silages were analysed before ensiling on day 0 and after 1, 2, 3 and 7 days of ensiling (Table 3-1).

Table 3-1: Lucerne forage and silage pH changes in 250 ml and 5 L silos for day 0, 1, 3 and 7

Fermentation Time	250 ml silo		5 litre silo	
	T	C	T	C
Day 0 mean (SEM)	5.4 (0.00) ^a	5.5 (0.00) ^b		
Day 1 mean (SEM)	5.3 (0.00) ^c	5.4 (0.00) ^d		
Day 3 mean (SEM)	5.7 (0.03) ^e	5.7 (0.06) ^f	5.6 (0.03) ^h	5.6 (0.00) ^h
Day 7 mean (SEM)	5.8 (0.00) ^g	5.8 (0.00) ^g		

SEM= standard error mean, means with different superscripts between T and C are significant, $P < 0.05$, mean values are for 3 analyses

The nutrient composition and pH changes of both *Lactobacillus plantarum* MTD-1 treated and control lucerne silages before and after 90 days of ensiling were chemically analysed as represented in Table 3-2.

Table 3-2: Lucerne forage and silage pH and nutrient composition

	Lucerne forage		Lucerne silage	
	Before ensiling day 0		After ensiling day 90	
	Control (C)	Treatment (T)	Control (C)	Treatment (T)
Mean nutrient composition	Mean (SEM)	Mean (SEM)	Mean (SEM)	Mean (SEM)
pH	5.5 (0.06) ^c	5.4 (0.06) ^b	5.4 (0.03) ^b	5.3 (0.00) ^a
DM (g/kg DM)	282.8 (0.70) ^e	276.8 (0.95) ^a	278.0 (2.26) ^a	273.3 (0.63) ^a
Crude Protein (g/kg DM)	234.3 (2.33) ^a	276.8 (0.95) ^a	239.5 (1.32) ^a	273.3 (0.63) ^a
Ether Extract (g/kg DM)	26.1 (0.22) ^c	27.1 (0.14) ^b	29.9 (0.68) ^a	30.7 (0.70) ^a
Ash (g/kg DM)	165.0 (1.00) ^c	162.7 (2.36) ^b	186.2 (3.68) ^a	188.7 (2.75) ^a
NDF (g/kg DM)	323.3 (4.67) ^c	314.7 (3.42) ^b	342.5 (2.66) ^a	349.8 (6.75) ^a
WSC (g/kg DM)	23.3 (2.53) ^c	20.2 (1.82) ^b	2.0 (0.63) ^b	6.1 (1.54) ^a
ME (MJ/kg DM)	9.8 (0.04) ^c	9.7 (0.06) ^b	9.4 (0.08) ^a	9.3 (0.09) ^a

Control D 0= Control Day 0 (before ensiling), LP MTD-1 D0 = *Lactobacillus plantarum* MTD-1 Day 0 (before ensiling), control D 90 = Control Day 90 (after 90 days of ensiling), LP MTD-1 D 90 = *Lactobacillus plantarum* Day 90 (after 90 days of ensiling), treatment silages with same superscripts in the same row are not significant ($P > 0.05$), treatment silages with different superscripts in the same row are significant ($P < 0.05$), control silages with same superscripts are not significant ($P > 0.05$), control silages with different superscripts are significant ($P < 0.05$), $n=8$

In this study, the total DM content of the silages for control silage, after 90 days of ensiling decreased ($P < 0.05$) from 282.8 to 278.0 g/kg DM. The pH for *Lactobacillus plantarum* MTD-1 treated silages declined ($P < 0.05$) from

5.4 to 5.3, whereas the pH for the control silages decreased from 5.5 to 5.4, respectively. There were no significant differences in the crude protein content of the silages. .

The WSCs decreased ($P<0.05$) by 91.4 and 69.8 % for control and *Lactobacillus plantarum* MTD-1 treated silages, respectively. The metabolisable energy for the control and *Lactobacillus plantarum* MTD-1 treated silages was both reduced ($P<0.05$) by 4.1% but there were no significant differences between treatments

Table 3-3: Fermentation characteristics of LP MTD-1 Treated and Control silages

Fermentation characteristic	Lucerne silage	
	T Mean (SEM)	C Mean(SEM)
pH	5.3 (0.00) ^a	5.4 (0.03) ^b
Lactic acid (g/kg DM)	3.2 (0.12) ^a	7.4 (2.85) ^b
Acetic Acid (g/kg DM)	22.9 (4.40) ^a	19.5 (3.07) ^a
Propionic Acid (g/kg DM)	8.2 (4.53) ^a	6.2 (5.48) ^b
Butyric Acid (g/kg DM)	0.1 (0.06) ^a	0.1 (0.05) ^a
Ethanol (g/kg DM)	1.4 (0.20) ^a	2.1 (0.20) ^b
NH ₃ -N (g/kg DM)	1.6 (0.40) ^a	1.1 (0.03) ^a

Mean (SD), NH₃-N= Ammonium nitrogen, mean values with same superscripts are not significant ($P>0.05$), mean values with different superscripts are significant ($P<0.05$), n=7

The final silage fermentation characteristics are presented in Table 3-3. The *Lactobacillus plantarum* MTD-1 treated silages resulted in lower ($P<0.05$) pH values (5.3) than control silages after 90 days of ensiling. The *Lactobacillus plantarum* MTD-1 treated silage had lower ($P<0.05$) ethanol content of 1.41 g/kg DM than the control silages, which produced 2.14 g/kg DM after 90 days of ensiling, representing a 51.8 % increase in the control silages. There was no significant difference in total butyric acid or ammonia concentration

The pH of both *Lactobacillus plantarum* MTD-1 and control silages was plotted on linear graph to analyse the trend in the pH change with time, from day 0 to day 90 (Figure 3-2). The pH steadily increased in both treatments

up to 5.8 after seven days of fermentation. However, after 90 days of fermentation the pH decreased there were no significant differences in pH between treatments.

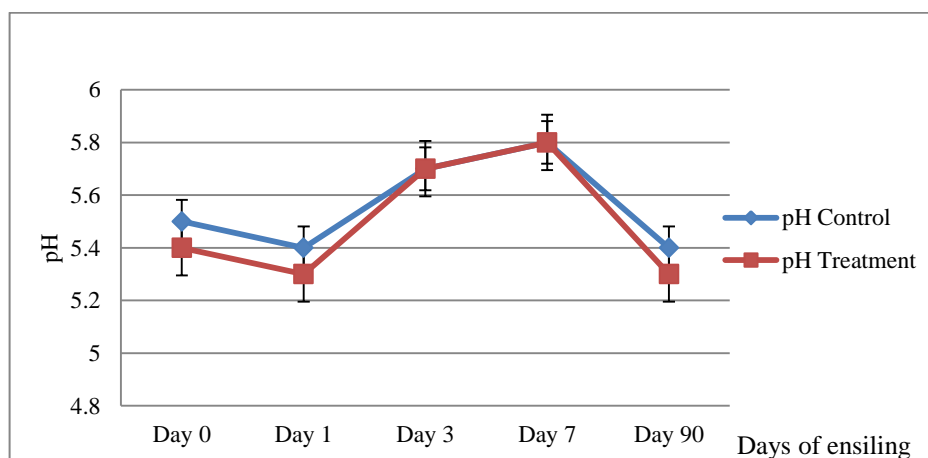


Figure 3-2: The pH changing trend of lucerne silage treated with LP MTD-1 against Control silages

The temperature of the *Lactobacillus plantarum* MTD-1 treated silages was lower ($P < 0.05$) than control silages after 1 day of being exposed to air. The mean temperature rise for both control and *Lactobacillus plantarum* MTD-1 silages were 6.7°C and 4.2°C over 8 consecutive days of exposing the silages to air. The temperatures of the silages steadily increased from the first day of exposure with increased ($P < 0.05$) temperature changes in the control silages compared to the *Lactobacillus plantarum* MTD-1 treated silages. The temperature of the silages was measured every hour for eight days and the aerobic stability of the control and treated/inoculated silages in the form of temperature rise were stable (remained below 3°C) for the entire period (Figure 3-3). The temperatures of both control and *Lactobacillus plantarum* MTD-1 treated silages were above the ambient temperature of 5.5°C but below 8.5°C by day 8 (Figure 3-3). However, the control silages had higher ($P < 0.05$) temperature rises from the beginning of temperature measurement and heated up above ambient temperature within 24 hours (1 day), whereas the treated silage temperature rose above ambient temperature after 180 hours (7.5 days). The control silages were almost 1.0°C below ($P < 0.05$) the aerobic stability temperature line of 8.5°C whereas the

Lactobacillus plantarum MTD-1 treated silages temperature was at ambient temperature (5.5°C) by day 8 (Figure 3-3).

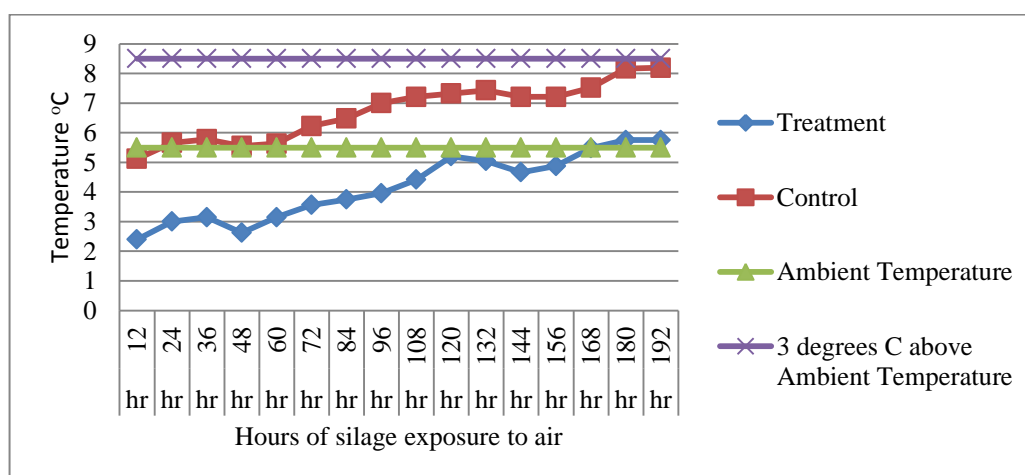


Figure 3-3: Silage aerobic stability / temperature rises at every 12 hour period

3.4 Discussion

The nutrient composition of both control and *Lactobacillus plantarum* MTD-1 silages was altered after 90 days of ensiling, resulting in an increased crude protein, crude fat (ether extract), mineral (ash) and NDF composition, whereas WSCs and metabolisable energy content decreased in both control and treated silages. The increase in the nutrient contents, such as crude protein, NDF, ether extract and minerals, and the decrease in silage DM content can be explained by the decrease in WSCs (Wattiaux, 1999; Kung and Der Bedrosian, 2010).

The metabolisable energy (ME MJ/kg DM) content for both control and *Lactobacillus plantarum* MTD-1 inoculated silage decreased by 4.1% after 90 days of fermentation. The loss in forage silage metabolisable energy can be attributed to the loss of forage carbohydrates in the form of WSCs and the degradation of forage cell walls during fermentation (Dewar *et al.*, 1963).

The crude protein content in the control and *Lactobacillus plantarum* MTD-1 treated samples increased by 2.2 and 2.5 % respectively after 90 days of ensiling. The increase in crude protein content could be attributed to the

proteolysis that occurs in lucerne fermentation because of high pH values, which favour the growth of clostridial bacteria resulting in both high concentration of ammonia-N (Wattiaux, 1999; Kung, 2010) as well as the loss of WSCs which occur during fermentation (Morrison, 1988; Kung and Der Bedrosian, 2010). Fernandez and O'kiley (2008) ensiled difficult-to-ensile forage crops like lucerne using *Lactobacillus plantarum* MTD-1 in which crude protein content was increased by 1.2 % compared to control silages. Similarly, Kung *et al.* (1991b) used *Lactobacillus plantarum* MTD-1 to ensile lucerne and found that crude protein increased by 2.8 % compared to fresh lucerne crops.

The increase in the crude fat (ether extract), ash and neutral detergent fibre in both control and *Lactobacillus plantarum* MTD-1 treated silages can be explained by the loss of WSCs, which is a substrate for LAB during fermentation (Wattiaux, 1999). The crude fat content of the silages in both the control and *Lactobacillus plantarum* MTD-1 treated silages increased by 14.6 and 13.3%, respectively after 90 days of fermentation. The total mineral content of the final silage increased by 12.8 and 16.0% for the control and treated silages, whereas the NDF of both control and *Lactobacillus plantarum* MTD-1 treated silages increased by 5.9 and 11.2% respectively after 90 days of ensiling. *Lactobacillus plantarum* MTD-1 treated silage resulted in a higher increase in both mineral and NDF and a decrease in crude fat content than in control silages.

The inoculated silages had a pH of 5.3 whereas the control silages had a pH of 5.4 after 90 days of ensiling. The increase in pH for both treated and control silages after 24 hours could be attributed to the lucerne high buffering capacity, which encourages the growth of clostridia bacteria that breaks down proteins, releasing NH₃-N resulting in early silage fermentation high pH values (Kung *et al.*, 1991b). The high pH value of the treated silages could also be explained by the prolonged fermentation, which resulted in the conversion of lactic acid to acetic acid after 90 days of ensiling (Kung and Der Bedrosian, 2010).

Wattiaux (1999) reported that in ensiling of lucerne forages pH changes do not decrease below 4.5. Jones *et al.* (1992), in ensiling of 290 g/kg DM content lucerne with *Lactobacillus plantarum* and *Pediococcus cerevisiae*, achieved a pH decrease of 5.08 and 4.92 after 64 days of fermentation. The silages were ensiled at 2×10^2 colony forming units per gram and 6×10^6 colony forming units per gram forage, representing low and high ensiling rates (Jones *et al.*, 1992). In this study, the *Lactobacillus plantarum* MTD-1 was applied at 1×10^6 cfu per gram lucerne lower than Jones *et al.* (1992) high inoculants application rate to achieve a pH of 5.3. Therefore the difference in pH values between this study and Jones *et al.* (1992) study could be attributed to the difference in the colony forming units' concentration. Kung *et al.* (1991b) ensiled lucerne at 300 g/kg DM content with Ecosyl™ *Lactobacillus plantarum* at 30° C to obtain a pH value of 4.5 after 45 days of ensiling. In this study, the ensiling was conducted at 5.5° C with relatively lower DM forages of 282.8 and 276.8 g/kg DM than that of Kung *et al.* (1991b). The difference in pH values between Kung *et al.*, (1991b) and this study could be attributed to the difference in ensiling temperatures and dry matter content of the forages at ensiling.

The decrease in lactic acid production in this study as compared to Kung *et al.* (1991b) study could be attributed to the high buffering capacity of lucerne which resulted in a high pH, encouraging the proliferation of clostridia bacteria and inhibition of the growth of LAB (Dinić *et al.*, 2010b). The high increase in the growth of clostridia bacteria increases both the proteolysis of proteins and a high production of butyric acid and ammonia favouring the growth of acetic bacteria (Dinić *et al.*, 2010b; Kung, 2010). The difference in the ensiling duration could also contribute to the declined of more lactic in this study compared to Kung *et al.* (1991b) study.

Whiter and Kung (2001) inoculated lucerne forages at 300 g/kg DM content for 45 days with *Lactobacillus plantarum* MTD-1 in which the acetic acid content was 26.7g/kg DM and lactic acid concentration was at 78.9 g/kg DM. In this study, the lactic acid content of the treated silage after 90 days was 3.19 g/kg DM whereas the acetic acid concentration was at 22.94 g/kg DM.

Therefore in this study, it can be concluded that lactic acid in treated silages was converted to acetic acid through the effects of the *Lactobacillus plantarum* MTD-1 inoculant activities because the forages were fermented for a long period of time (90 days).

Lactobacillus plantarum MTD-1 treated silage resulted in a marginally higher butyric acid content of 0.07 g/kg DM against 0.06 g/kg DM for the control silages. In the *Lactobacillus plantarum* MTD-1 lucerne ensiling study of Fernández and O'kiely (2008), a low butyric acid concentration of 0.06 g/kg DM was realised as compared to 0.29 g/kg DM in the control silages. In this study an almost equal concentration of butyric acid of 0.06 g/kg DM and 0.07 g/kg DM for treated and control silages was produced as compared to Fernández and O'kiely (2008) results. Jonsson (1991) stated that high concentrations of butyric acid in silages indicate poor fermentation due to moulds, spores and clostridial bacteria fermentation. Kung (2010) reported that a butyric acid concentration in excess of 5 % silage DM content signals silage clostridial fermentation. However, in both treated and control silages, too little butyric acid of (7.0×10^{-3}) % and (6.0×10^{-3}) % DM respectively was realised to be attributed to clostridial fermentation.

Whiter and Kung (2001) ensiled lucerne silages at 300 g/kg DM for 45 days using *Lactobacillus plantarum* MTD-1 to obtain a silage with $\text{NH}_3\text{-N}$ concentration of 2.63 g/kg DM more than the $\text{NH}_3\text{-N}$ concentration obtained in both treated and control silages. Kung (2010) stated that in silage fermentation characteristics, a high concentration of $\text{NH}_3\text{-N}$, in the range of 120-150 g/kg of crude protein, is attributed to high degree of forage proteolysis due to clostridial and enterobacterial fermentation. Silages which have undergone clostridial fermentation have $\text{NH}_3\text{-N}$ concentration greater than 100 g/kg DM with more butyric acid concentration than lactic acid, as well as having strong smell (Wattiaux, 1999). In both treated and control silages 1.61g/kg and 1.13g/kg DM of $\text{NH}_3\text{-N}$ and more lactic acid (3.19 and 7.38 g/kg DM) than total butyric acid (0.07 and 0.06 g/kg DM) was realised, ruling out the possibility of both clostridial and enterobacterial fermentation. However on opening the silos, the control silages had a strong

pungent/smell which could be attributed to both enterobacterial and clostridial fermentation.

The treatment of the forage crops with *Lactobacillus plantarum* MTD-1 increased propionic acid concentration compared to the control silages after 90 days of ensiling. The treated silages produced 8.15 g/kg DM propionic acid whereas the control silages had 6.19 g/kg DM. Fernández and O'kiely, (2008) ensiled lucerne using *Lactobacillus plantarum* MTD-1 in which 7.7 g/kg DM propionic acid was produced and 7.6 g/kg DM was produced in control silages. Kung (2010) reported that in high quality silages, which have undergone good fermentation, propionic acid concentration is almost negligible. However, silages with a high pH contain propionibacteria which convert glucose and lactic acid to propionic acid (Kung, 2010). In low quality silages with the presence of clostridia bacteria, propionic acid is in the range of 3-5 g/kg DM (Kung, 2010). In this study, higher concentrations of propionic acids than the range of 3-5 g/kg DM were produced in both treated and control silages, indicating the presence of either propionibacteria or clostridia bacteria. However, the low levels of both butyric acid and NH₃-N produced in this study rules out the possibility of the presence of clostridia bacteria but propionibacteria. The presence of propionibacteria in the silage was not verified/ tested.

The lower temperature reading of the *Lactobacillus plantarum* MTD-1 treated silages than the control silages after 1 day of being exposed to air indicated that there were less micro-organism in the treated silages than in control silages. This is because during the stable phase of the ensiling process all the oxygen is assumed to have been used up and all biological activities in the silo are inhibited (Barnhart and Nadeau, 2008). Therefore the higher temperature reading of the control silages at day 1 of measuring aerobic stability could be attributed to the growth of more anaerobic bacteria such as clostridia because of high pH values than in treated silages (Wattiaux, 1999).

The aerobic stability of the *Lactobacillus plantarum* MTD-1 treated silage was more stable than the control silages during the eight days of exposure to

air. The higher aerobic stability of the treated silages compared to the control silages could be attributed to the high concentration of acetic acid (Jatkauskas and Vrotniankiene, 2009; Kung, 2010) and propionic acid, which is antifungal (Merry and Davies, 1999) in treated silages after 90 days of ensiling. It is reported that a high concentration of lactic acid in silage fermentation results in reduced aerobic stability during feedout, hence the low aerobic stability in the control silages (Jatkauskas and Vrotniankiene, 2009; Driehuis *et al.*, 1999).

However, although the *Lactobacillus plantarum* MTD-1 treated and control silages were aerobically stable during exposure to air for eight days, the treated silages were more stable than the control silages because the control silage started heating up above ambient temperature after 24 hours, whereas the treated silages rose above the ambient temperature after 7 days. Kung *et al.* (1991b) in the ensiling of lucerne silages with Ecosyl™ *Lactobacillus plantarum* using pH as a criteria for establishing aerobic stability, found that the treated silage pH started increasing after four days of exposure, indicating proliferation of microbes which increase silage temperature and pH (Barnhart and Nadeau, 2008; Woolford, 1984). In the fermentation of lucerne forage using Ecosyl™ *Lactobacillus plantarum*, Fernandez and O'Kiely (2008) in Ireland, observed that the maximum temperature rise by day five in the aerobic stability of lucerne was 25° C for the treated silages and 22° C for the control silages, whereas in this study by day five the maximum temperature rises for treated and control silages were 5.0° C and 7.3° C, respectively.

The silage was ensiled at 282.8 g/kg DM and 276.8 g/kg DM for both control and treatment samples respectively, against the recommended 30-35% DM content. However, *Lactobacillus plantarum* MTD-1 inoculants proved that it is effective even at low forage DM content, like less than 300 g/kg DM, to improve silage quality characteristics such as aerobic stability (Heron and Owen, 2010).

Despite the two silages, from both *Lactobacillus plantarum* MTD-1 treated silages and control silages having almost same final pH levels of 5.3 and 5.4 after 90 days of ensiling, treated silages had better aerobic stability than the control silages. This finding agrees with Heron and Owen (2010) who reported that Ecosyl™ 100 *Lactobacillus plantarum* MTD-1 inoculant is effective over a wide range of pH.

3.5 Conclusion

In this study the inoculation of lucerne forages with *Lactobacillus plantarum* MTD-1 improved aerobic stability after the silages have been opened and exposed to air. The results have demonstrated that *Lactobacillus plantarum* MTD-1 biological inoculant is effective in ensiling lucerne forages.

4. Chapter Four: Experiment 3 A desk top study to evaluate the potential effects of including dried lucerne in dairy cows' total mixed ration (TMR) on enteric methane emission using published equations

4.1 Introduction

Enteric methane production from ruminants is a major contributor to greenhouse gases (Alluwong *et al.*, 2011). Dairy cows, as ruminant livestock, use pregastric fermentation in digesting forages to convert the forages to useful nutrients (Mirzae-Aghsaghali and Maheri-Sis, 2011). The feeding of dairy cows with a high proportion of forages results in loss of dietary energy in the form of enteric methane (Johnson and Johnson, 1995). Johnson *et al.* (1993) stated (in Johnson and Johnson, 1995) that this dietary energy loss is in the range of 20-120 J/kJ of gross forage energy intake. However, the use of high quality forages such as lucerne (*Medicago sativa, sativa.*) with low fibre and high soluble carbohydrates results in reduced enteric methane emissions (Beuchemin *et al.*, 2007; Ulyatt and Lassey, 2001). The feeding of dairy cows on maize silage-based total mixed ration (TMR) has been one of the most effective strategies in reducing enteric methane emission (Beuchemin *et al.*, 2007; Vellinga and Hoving, 2011; Tamminga *et al.*, 2007). Maize silage has a high proportion of starch with high rumen degradation (Mayne and O'Kiely, 2005; Tamminga *et al.*, 2007) which results in reduced enteric methane emissions (Beuchemin *et al.*, 2007; Tamminga *et al.*, 2007). Increasing the nutrient quality of the forages, such as with maize silage, improves voluntary dry matter intake (DMI) in dairy cows and this reduces forage rumen retention time, resulting in a reduced proportion of dietary energy being converted to methane (Blaxter and Clapperton, 1965). It is reported that an increase in diet ruminal passage rate due to increased feed intake level results in low rumen degradable substrate, both increasing the escape of rumen digesta substrate (products of digestion which leave the rumen) and leading to a decrease in enteric methane emissions (Tamminga *et al.*, 2007, Benchaar *et al.*, 2001). Increasing dry matter intake (DMI) in

dairy cows in the form of feeding levels reduces enteric methane as a proportion of gross dietary energy intake (Boadi *et al.*, 2004). Improving milk production by feeding dairy cows on high quality diets increases both dry matter intake (DMI) and enteric methane emissions but results in less enteric methane emissions when the emissions are expressed as the proportion of total milk production (Ulyatt and Lassey, 2001). However, although increased use of forage-based feed increases enteric methane emission in dairy cows, it is expected that the inclusion of lucerne silage instead of a grass-based diet in dairy cow total mixed ration will reduce methane production as well as improving the cows' milk production because of lower fibre content and reduced rumen digesta retention time (Beuchemin *et al.*, 2007; Dewhurst *et al.*, 2009).

In this study two dairy cow total mixed rations were formulated containing grass silage, maize silage, concentrate feed and dried lucerne in order to predict methane emissions using regression equations. The study aimed to evaluate the effect of including dried lucerne forage by replacing maize silage in forage-based dairy cow total mixed ration (TMR) on the loss of dietary energy in the form of enteric methane. It was expected that the two TMR would either have similar enteric methane emissions or the inclusion of dried lucerne would produce lower methane emissions than maize silage.

4.2 Materials and Methods

Two types of dairy cow total mixed ration (TMR) were formulated, using Scottish Agricultural College (SAC) FeedByte, containing maize silage and dried lucerne along with grass silage. The SAC (2006) states (in Chagunda *et al.*, 2010a) that SAC FeedByte is a model for simulating the physical processes based on lowest-cost diet formulation and linear programming modelling. The maize silage based ration contained grass silage and maize silage in the ratio of 2.7:0.5, 3.3:0.4 and 3.9:0.3, respectively to produce 20, 25 or 30 kg milk per day. The dried lucerne based ration contained grass silage, concentrates supplement and dried lucerne in the ratio of 2.1:0.2: 1.1, 2.4: 0.3: 1.3 and 2.3:0.3:1.8 to produce 20, 25 or 30 kg milk per day. The first

three rations (High Maize Silage Forage, Medium Maize Silage Forage and Low Maize Silage Forage) contained maize silage with lower levels of inclusion as milk production was increased than grass silage. The second three rations (Low Lucerne Forage, Medium Lucerne Forage, and High Lucerne Forage) contained dried lucerne forage as a substitute for maize silage. The amount of lucerne being incorporated in the ration was increased in line with the increasing total milk yield per day produced by the cow. The nutrient quality of the feeds were for the grass silage, maize silage, concentrate and dried lucerne respectively: ME (MJ/kgDM) 10.6,11.0,13.0,8.8 : CP (g/kgDM) 150,90,210,180. The detailed feed nutrient compositions of the two TMRs are included in appendix 2. The amount of enteric methane emission in dairy cows is inversely proportional to the amount of feed grains offered (Boadi *et al.*, 2004). Maize silage is a high quality dairy cows feed which results in both higher milk production and lower enteric methane emission than grass silage because of the high concentration of starch (Tamminga *et al.*, 2007). Therefore the reduction in the proportion of maize silage in TMR could not reduce milk production from the cows. Similarly, lucerne as legume forages have higher nutrient quality than grass silage resulting in high milk production and low enteric methane emission compared to grass silage (Tamminga *et al.*, 2007).

The rations were formulated based on a standard 600 kg dairy cow producing 20, 25 or 30 kg milk per day, with milk protein and milk fat concentrations of 34 and 39 g/kg, respectively. The model produced the ration for a dairy cow in the 40th week prior to calving with a 2.5 body conditional score (Mulvaney, 1977) and 12 weeks into lactation period.

The required enteric methane emission prediction input variables, for the published equations, from the formulated total mixed rations (TMRs) are presented in Table 4-1. Maize silage usually contains some concentrates in the form of maize grain and therefore there was no need for concentrate supplementation in maize silage TMR but in lucerne TMR additional concentrate feed was required to meet the dairy cow energy requirements.

Table 4-1: Feed nutrient composition, cow DMI, feed forage content and milk yield per day for methane emission prediction

		Methane prediction variables		equation input				
TMR	DMI kg	Forage kg	CDMI g	Milk yield/d (kg)	CP (g)	NDF diet	g/kg	ME(MJ/kg DM)
HMS								
F	16.0	16.0	-	20.0	141	463		10.7
MMS								
F	18.5	18.5	-	25.0	144	462		10.6
LMSF	21.0	21.0	-	30.0	146	461		10.6
LLF	16.8	15.9	0.87	20.0	163	464		10.1
MLF	19.4	18.1	1.30	25.0	164	463		10.2
HLF	22.3	20.6	1.70	30.0	167	452		10.1

HMSF= High maize silage forage, MMSF= medium maize silage forage, LMS= Low maize silage forage, LLF = Low lucerne forage, MLF = medium lucerne forage, HLF = High lucerne forage, CP= Crude protein, DMI= Dry matter intake, CDMI = concentrate dry matter intake, NDF = neutral detergent fibre, ME = metabolisable energy, kg = kilogram, MJ/ kg = mega joules per kilogram,

The enteric methane emissions that would be expected had the cows been fed the formulated TMR were estimated using regression equations developed by Yates *et al.*, (2003), Mills *et al.*, (2003) (linear and nonlinear equations), Yan *et al.*, (2005), Kriss, (1930) and Axelsson, (1949) as presented in Table 4-2. These equations were used in this study because they are frequently used in dietary methane emission predictions studies such as Bell *et al.*, (2009) and Wilkerson *et al.*, (1995). The formulated TMRs quality characteristics also contained their input variables.

Table 4-2: Statistical equations used in predicting methane production

Prediction equation	Source
$CH_4 \text{ (MJ/d)} = 1.36 + 1.21 \times DMI - 0.825 \times CDMI + 12.8 \times NDF$	Yates <i>et al.</i> , (2003)
$CH_4 \text{ (MJ/d)} = 8.25 + 0.07 \times MEI \text{ (MJ/d)}$	Mills <i>et al.</i> , (2003)
$CH_4 \text{ (MJ/d)} = 45.89 - (45.89 + 0) \times e^{(-0.003 \times MEI \text{ MJ/d})}$	Mills <i>et al.</i> , (2003)
$CH_4 \text{ (MJ/d)} = (47.82 \times DMI \text{ (kg/d)} - 0.762 \times DMI \times DMI - 41) \times 0.03954$	Yan <i>et al.</i> , (2005)
$CH_4 \text{ (kg/d)} = (75.42 + 94.28 \times DMI \text{ (kg/d)}) \times 0.05524$	Kriss, (1930) ^a
$CH_4 \text{ (MJ/d)} = (18 + 22.5 \times DMI) \times 0.05565 \text{ (MJ/g } CH_4)$	Kriss, (1930) ^b
$CH_4 \text{ (MJ/d)} = -2.07 + 2.636 \times DMI \text{ (kg/d)} - 0.105 \times DMI \times DMI \text{ (kg/d)}$	Axelsson, (1949)

The enteric methane emissions were calculated using seven different equations which were selected based on the availability of the required input variables from the formulated TMRs as well as being the most frequently used equations in enteric methane prediction studies (Table 4-2). Bell *et al.* (2009) evaluated the suitability of a number of enteric methane emission prediction regression equations for predicting the methane emissions of high milk yielding Langhill cows, including some of the equations used in this study. The basis for enteric methane emission estimation in dairy cows is either feed DMI or feed metabolisable energy intake (Mills *et al.*, 2003). Enteric methane emission empirical equations formulated on DMI, gross energy intake and metabolisable energy intake ought to be nonlinear, because emissions decrease with increased intake of these dietary characteristics (Mills *et al.*, 2003). The nonlinear methane emission prediction MEI based empirical equation developed by Mills *et al.* (2003) was recommended for the methane emission prediction of Langhill cows (Bell *et al.*, 2009). Axelsson (1949) methane emission prediction equation, which uses DMI as its input variables, underestimates methane emissions when used on high DMI cows because emissions start decreasing when DMI is in excess of 12.5 kg DM per day (Wilkerson *et al.*, 1995; Mills *et al.*, 2003). The methane prediction equations of Axelsson, (1949) and Kriss, (1930) equation a) respectively underestimate and overestimate enteric methane emission in dairy cows comparing with other work done elsewhere. The enteric methane estimates from Axelsson, (1949) and Kriss, (1930) equation a) were therefore excluded in estimating the total mean, minimum and maximum enteric methane emission of the two TMRs.

The predicted enteric methane emissions were analysed by using Excel to evaluate the difference in the emission trend of enteric methane of the high forage TMR feed containing dried lucerne forage and the maize silage based formulated total mixed rations.

4.3 Results

The predicted enteric methane emissions from the two formulated TMRs by using the five regression equations are presented in Table 4-3.

Table 4-3: Predicted enteric methane emission for best five equations from maize and dried lucerne based feed at 20, 25 and 30 kg milk yield per day

Equations	Maize based lucerne TMR		
	HMSF	MMSF	LMSF
	20 kg milk	25 kg milk	30 kg milk
Yates <i>et al.</i> , (2003), CH_4 (MJ/d) = $1.36 + 1.21 \times DMI - 0.825 \times CDMI + 12.8 \times NDF$	21.3	24.3	27.4
Mills <i>et al.</i> , (2003), CH_4 (MJ/d) = $8.25 + 0.07 \times MEI$ (MJ/d)	20.2	22.0	23.8
Mills <i>et al.</i> , (2003), $45.89 - (45.89 + 0) \times e(-0.003 \times MEI$ MJ/d)	18.4	20.4	22.4
Yan <i>et al.</i> , (2005), CH_4 (MJ/d) = $(47.82 \times DMI$ (kg/d) – 0.762 x DMI x DMI-41) x 0.03954	20.9	23.1	24.8
Kriss (1930) ^b , CH_4 (MJ/d) = $(18+22.5 \times DMI) \times 0.05565$ (MJ/g CH_4)	21.0	24.2	27.3
Average CH_4	20.4	22.8	25.1
	Dried lucerne based TMR		
	LLF	MLF	HLF
Yates <i>et al.</i> , (2003), CH_4 (MJ/d) = $1.36 + 1.21 \times DMI - 0.825 \times CDMI + 12.8 \times NDF$	21.6	24.4	27.5
Mills <i>et al.</i> , (2003), CH_4 (MJ/d) = $8.25 + 0.07 \times MEI$ (MJ/d)	20.1	22.1	24.0
Mills <i>et al.</i> , (2003), $45.89 - (45.89 + 0) \times e(-0.003 \times MEI$ MJ/d)	18.3	20.5	22.5
Yan <i>et al.</i> , (2005), CH_4 (MJ/d) = $(18+22.5 \times DMI) \times 0.05565$ (MJ/g CH_4)	21.6	23.7	25.6
Kriss (1930) ^b , CH_4 (MJ/d) = $(18+22.5 \times DMI) \times 0.05565$ (MJ/g CH_4)	22.0	25.3	28.9
Average CH_4	20.7	23.2	25.7

HMSF= High maize silage forage, MMSF= medium maize silage forage, LMSF = low maize silage forage, LLF= Low lucerne forage, MLF= medium lucerne forage, HLF= High lucerne forage

Table 4-4: Total mean enteric methane, methane per DMI and methane per unit milk yield predicted from maize and dried lucerne based diet

Methane predicted at different milk output in MJ /day												
Milk output	20 kg				25 kg				30 kg / day			
Feed / TMR	Mean	Min.	SD	Max.	Mean	Min	SD	Max	Mean	Min	SD	Max
Maize silage	20.4	18.4	1.2	21.3	22.8	20.4	1.6	24.3	25.1	22.4	2.2	27.4
Dried lucerne	20.7	18.3	1.5	22.0	23.2	20.54	1.9	25.3	25.7	22.54	2.6	28.9

Methane prediction per unit DMI in MJ/ day												
Maize silage	1.27	1.15	0.07	1.33	1.23	1.42	0.10	1.31	1.20	1.06	0.10	1.30
Dried lucerne	1.23	1.09	0.07	1.31	1.20	1.06	0.10	1.50	1.15	1.01	0.12	1.30

Methane prediction per unit milk yield / day in MJ / day												
Maize silage	1.02	0.92	0.06	1.07	0.91	0.82	0.07	0.97	0.84	0.75	0.07	0.91
Dried lucerne	1.04	0.92	0.08	1.10	0.93	0.82	0.08	1.01	0.86	0.75	0.09	0.96

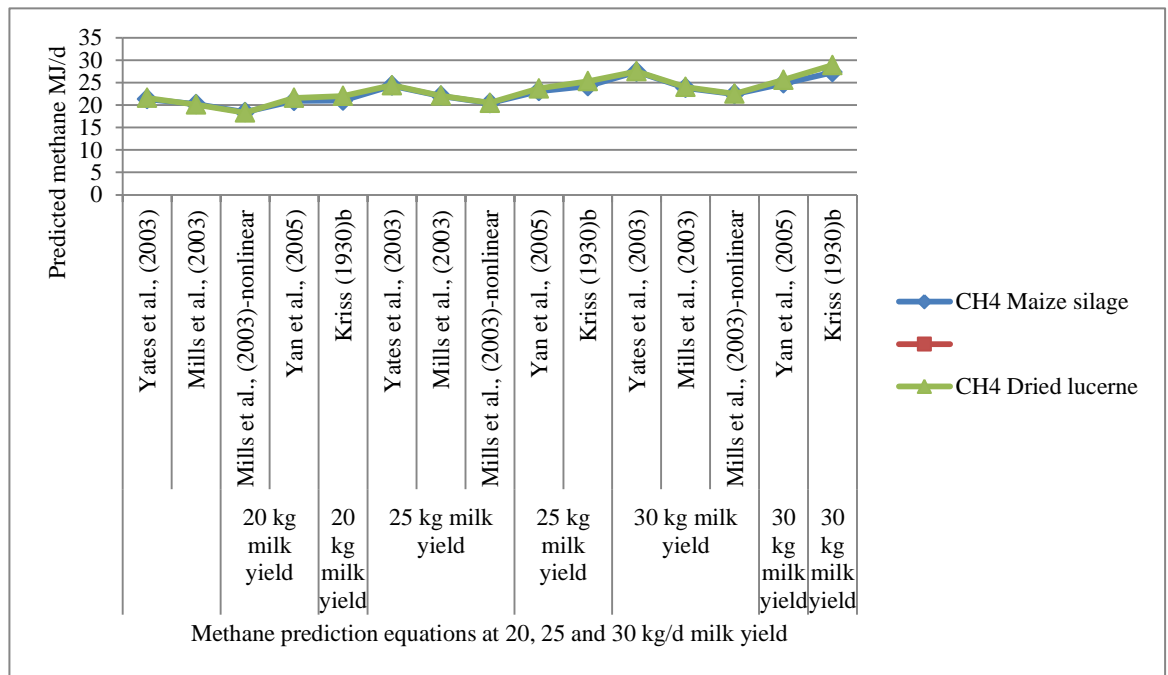


Figure 4-1: Trend of predicted absolute enteric methane emission at 20, 25 and 30 kg milk yield per day

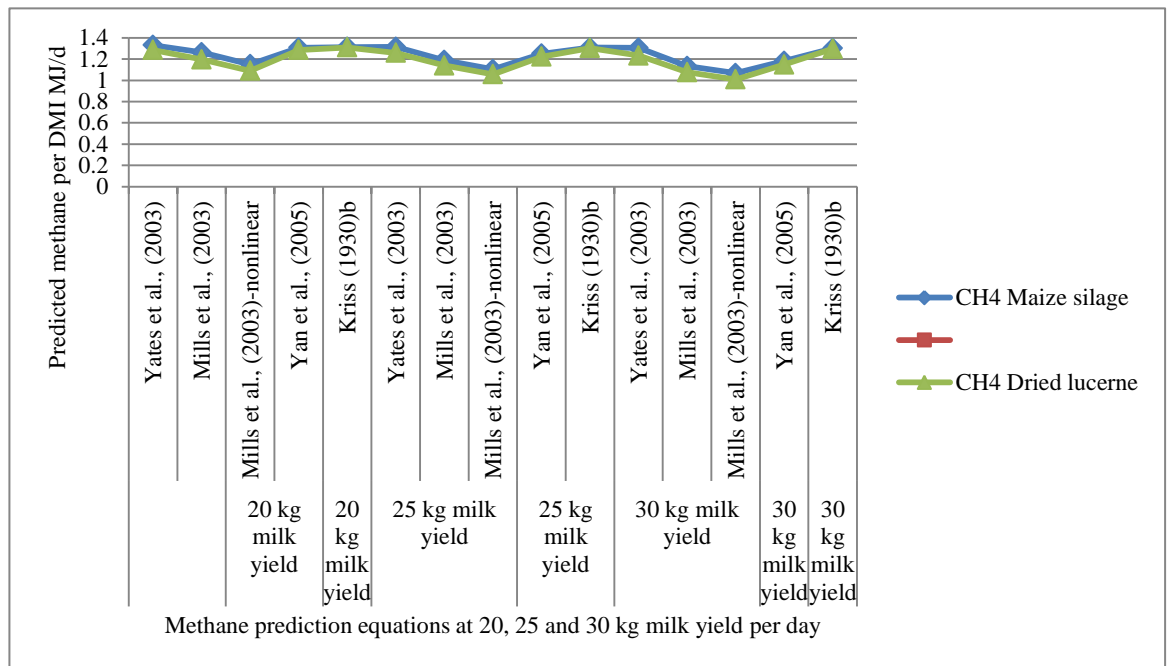


Figure 4-2: Trend of predicted enteric methane emission per unit DMI at 20, 25 and 30 kg milk yield

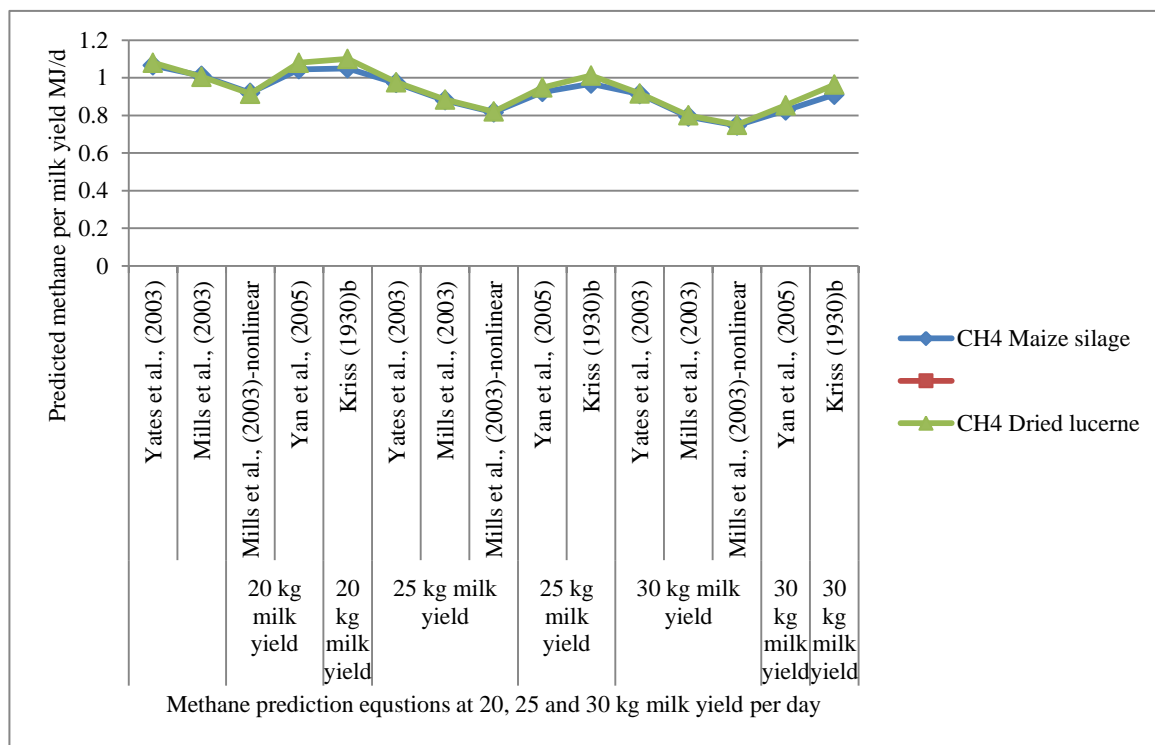


Figure 4-3: Trend of predicted enteric methane emission per unit milk yield at 20, 25 and 30 kg milk yield

4.3.1 Effect of diet dry matter intake on methane production using equations

The predicted enteric methane (CH₄) concentrations, in Table 4-4 and Figure 4-1, indicated that increasing DMI in the cows would increase methane emissions. In maize silage based feed, the reduction in the proportion of the maize silage in the ration increased the proportions of the grass silage in the feed, which resulted in increased grass DMI in cows as the milk production increased from 20 to 30 kg/d (Table 4-4). This increase in animal grass silage DMI resulted in increased absolute enteric methane emissions by 11.8 % and 10.1 %, respectively. In dried lucerne based diet, the inclusion of lucerne to produce 20, 25 and 30 kg milk yield per day increased the total enteric methane emission by 12.1 % and 10.8 %, respectively (Table 4-4). The inclusion of dried lucerne in maize silage TMR resulted in increased absolute enteric methane emissions. However, Figure 4-1 indicated that though the enteric methane emission increased with the inclusion of dried

lucerne, almost equal concentration of methane was emitted by both maize silage and dried lucerne based silage.

Increasing DMI in both maize silage and dried lucerne based diet resulted in reduced enteric methane emission per unit DMI. In maize silage based TMR, enteric methane emission was reduced by 3.1 % and 2.4 % from 20 kg/d milk production to produce 25 and 30 kg of milk per day respectively. In dried lucerne, enteric methane emission was reduced by 2.4 % and 4.2 % to produce 25 and 30 kg milk respectively. The enteric methane production reduction was increasing in dried lucerne based diet whereas in maize silage based diet was decreasing.

4.3.2 The effects of replacing maize silage with dried lucerne on methane emission

In this study, replacing maize silage with dried lucerne forages resulted in increasing total enteric methane emission by 1.5, 1.8 and 2.4 % respectively to produce 20, 25 and 30 kg milk per day. The increase in total enteric methane emission increased with increased inclusion of dried lucerne forage in the diet.

However, the enteric methane emission decreased when it was expressed per unit DMI (Table 4-4 and Figure 4-2). Enteric methane emission, as the proportion of DMI, decreased by 3.1, 2.4 and 4.2 %, respectively with increasing DMI to produce 20, 25 and 30 kg of milk per day. The reduction in enteric methane emission per unit DMI, increased with increased amount of dried lucerne added in the diet.

4.3.3 Effects of improved milk production on enteric methane emission when replacing maize silage with dried lucerne

The total predicted methane emissions increased with increasing milk production and dried lucerne in the TMR by 1.5, 1.8 and 2.4 % to produce 20, 25 and 30kg milk per day. The increase in methane production decreased with both increasing milk production and dried lucerne inclusion in the TMR. The enteric methane emission decreased in maize silage and

dried lucerne diet per unit milk yield by 10.8 % and 7.7 % and 18.4 and 7.5%, respectively to produce 25 and 30 kg milk per day. The reduction in enteric methane emission was higher in dried lucerne than in maize silage diet (Figure 4-3).

However, further analyses showed that the replacing of maize silage with dried lucerne, with increasing milk production, increased methane emission per unit milk yield by 2.0, 2.2 and 4.1 % to produce 20, 25 and 30 kg of milk with the inclusion of lucerne in the diet. The increase in methane emission per unit milk yield was diminishing with increasing dried lucerne inclusion in the diet.

4.4 Discussion

The mean enteric methane emissions, in maize silage based diet, for the five prediction equations, excluding the equations of Kriss (1930)^a and Axelsson (1930), are 21.31, 23.57 and 25.74 MJ/d for the production of 20, 25 and 30 kg milk per day. In the dried lucerne diet, the mean emissions in the same order are 21.92, 23.89 and 26.19 MJ/d. Crutzen *et al.* (1986) reported that the annual methane emission for dairy cows in the US and Europe, particularly in West Germany, was 84 and 95 kg respectively. The energy content of a unit kilogram of methane is almost equal to 55.65 MJ (Crutzen *et al.*, 1986). Therefore, converting the findings of Crutzen *et al.* (1986) on methane production as energy in mega joules per day, results in 12.8 and 14.5 MJ/d respectively. In a feeding trial aimed at measuring enteric methane emission from Swiss dairy cows at both maintenance and production levels, from a diet consisting of roughages and concentrate in equal concentration, Hindrichsen *et al.* (2005) reported methane emissions in the range of 461 to 600 L per cow per day. It is reported that in enteric methane emission, a loss of 1 L of methane as a result of methanogenic digestion represents a reduction of 39.5 kJ feed energy (Guan *et al.*, 2006). When the enteric methane emissions of 461 and 600 L per cow/day, are converted to feed energy, as MJ/day, they result in 18.2 and 23.7 MJ/cow/day respectively. EPA (1993) reported (in Johnson and Johnson,

1995) that annual methane emissions for dairy cows range from 109 to 126 kg, which translates to 16.6 and 19.2 MJ/day per cow. Bell *et al.* (2010) reported a mean enteric methane estimation of 25.06 MJ/d on the Langhill cows using the nonlinear equation of Mills *et al.* (2003), $\text{CH}_4 \text{ (MJ/kg)} = 45.89 + (45.89 + 0) \times e^{(-0.003 \times \text{MEI})}$. Bell *et al.* (2009) also reported the mean enteric methane emissions of 24.0, 22.3, 25.7, 25.1, 7.9 and 23.1 MJ/d from the data of the Langhill Holstein Friesian cows using both linear and nonlinear Mills *et al.* (2003), Yates *et al.* (2003), Kriss (1930), Axelsson (1949) and Yan *et al.* (2005) equations. Similarly, Ellis *et al.* (2007) predicted enteric methane emissions in dairy cows using Kriss (1930)^a, Axelsson (1949) and Mills *et al.* (2003) equations, in which the methane emission ranged from 4.31 to 17.20 MJ/kg respectively.

Enteric methane emissions for Yates *et al.*, (2003), Yan *et al.* (2005), and Mills *et al.* (2003), linear and nonlinear equations are comparable to those published by Crutzen *et al.* (1986), Bell *et al.* (2009) and Ellis *et al.* (2007). However, enteric methane emissions from Kriss (1930)^a and Axelsson (1949) equations are overestimated and underestimated, respectively compared to the emissions reported here. The ability of enteric methane emission prediction equations depends on the range of the data on which they were developed (Bell *et al.*, 2009). Kriss (1930)^a equation was developed from beef cattle methane emissions data, whereas Axelsson, (1949) equation was a result of methane emission data from both beef and dairy cattle (Yan *et al.*, 2009). In this study, the input variables could be greater than the data on which Kriss, (1930)^a equation was developed, resulting in enteric methane emission overestimation whereas for Axelsson (1949) increasing DMI to over 12.5 kg/d resulted in reducing methane emissions (Mills *et al.*, (2003). This means that Kriss (1930)^a statistical equation was developed on cows with low DMI.

Axelsson (1949) equations predicted enteric methane emissions which were decreasing as DMI increased, whereas the other equations' emissions increased with increasing DMI. In normal situations, enteric methane emissions increase with increasing total DMI (Lovett *et al.*, 2004).

Increasing DMI in the production of 20, 25 and 30 kg milk per day increased methane emission in both maize silage and dried lucerne based diets. This agrees with Johnson and Johnson (1995) and Blaxter and Clapperton (1965) who stated that increasing dry matter intake in ruminant livestock increases absolute enteric methane emission. In the feeding trial of dairy cows with whole lucerne diet, three-tenths lucerne and seven-tenths concentrate mixture, Benchaar *et al.* (2001) reported an increase in enteric CH₄ (Mcal/kg) emission with increasing DMI irrespective of diet species. Enteric methane emission in dairy cows fed legume forages is (Waghorn *et al.*, 2002) but not often (Van Dorland *et al.*, 2007) lower than emissions from grass fed ruminants. However, the absolute enteric methane emissions from maize silage and dried lucerne based diets were not significantly different with the latter resulting in reduced emissions.

Increasing DMI of both maize silage and dried lucerne based diets resulted in reduced enteric methane emissions per unit DMI to produce 20, 25 and 30 kg milk per day. In the maize silage based diet, enteric methane production per unit of DMI was reduced by 4.5 and 3.5 %, whereas in the dried lucerne based diet, emission was reduced by 5.4 and 4.9 %, to produce 25 and 30 kg milk from 20 kg milk yield per day. Johnson and Johnson (1995), Blaxter and Clapperton (1965) and Waghorn *et al.* (2002) stated that enteric methane emissions in ruminant livestock such as dairy cows, when expressed per unit DMI, decreases. In this study, comparing the methane production from the two diets, the dried lucerne diet had a higher decrease in methane production than maize silage based diet.

Replacing maize silage with dried lucerne in maize silage based ration, resulted in increased total enteric methane emissions which were not significantly different. This is in agreement with Johnson and Johnson (1995), Benchaar *et al.* (2001) and Blaxter and Clapperton (1965) who stated that increasing DMI in dairy cows increases enteric methane emission irrespective of forage/diet quality and species.

However, replacing maize silage with dried lucerne resulted in reduced enteric methane emissions of 2.3, 3.2 and 4.9 % per unit DMI, agreeing well with the fact that feeding dairy cows on lucerne legumes lowers methane emission (Beuchemin *et al.*, 2007), when emission is expressed as a proportion of total DMI (Johnson and Johnson, 1995). This is mainly because of increased ruminal digesta passage rate, which results in reduced duration for the association between feed and methanogens (Mathison *et al.*, 1998). The reduced association between methanogens and feed digesta in the rumen results in both reduced ruminal dietary fermentation rate and enteric methane emission (Mathison *et al.*, 1998). Enteric methane emission has a strong correlation with dietary rumen digestion (Kirchgessner *et al.*, 1995). McCaughey *et al.* (1999) reported that the inclusion of lucerne forage in beef cows' feed could reduce enteric methane emission by 10 %. Lower methane emission in dairy cows fed legume based forages such as lucerne is a result of many factors such as lower fibre content, increased DMI and reduced ruminal digesta retention time (Beuchemin *et al.*, 2007; Dewhurst *et al.*, 2009). Feed with a high proportion of legume forages decrease methane emission per unit kg DMI (Clark *et al.*, 2011). However, grass based dairy cow diet results in more enteric methane emissions per unit DMI than legume forage based diet (Ulyatt and Lassey 2001).

The total predicted enteric methane emissions increased (although not significantly) with increasing milk production for both maize silage and dried lucerne based diets. The enteric methane production increased by 2.9, 1.4 and 1.7 % as dried lucerne replaced maize silage to produce 20, 25 and 30 kg milk per day. Improving dairy cows' milk productivity slightly increases enteric methane emission (Johnson *et al.*, 1996). The total enteric methane emission increases because enhanced dairy cow productivity occurs under high DMI (O'Mara, 2004). Kirchgessner *et al.* (1995) reported an annual increase of 23 % methane emission in EU dairy cows by either feeding them on high grain based ration or by enhancing the cows' genetic merit, as milk production was increased from 5000 to 10,000 L. However, the increase in enteric methane production as maize silage was being replaced by dried

lucerne was not significant and the increasing emissions diminished with increasing dried lucerne proportions in the diet.

Replacing maize silage with dried lucerne, when absolute methane emissions were expressed as a proportion of milk yield, resulted in an insignificant increasing enteric methane production per unit milk yield. It is reported that improving the dairy cows' milk production reduces enteric methane emission per unit milk yield (Ulyatt and Lassey, 2001). This is because the enteric methane emission from maintenance processes is divided by the increased amount of milk produced (O'Mara, 2004). In Queensland, Australia, annual milk production was increased by 38% between the years 1988 to 1999, which resulted in 26 % enteric methane emission reductions per litre of milk produced (Howden and Reyenga, 1999). In this study, the non-significant marginal increase in enteric methane emissions could be attributed to the fact that both maize silage and dried lucerne are high quality forages (Beuchemin *et al.*, 2007; Johnson and Johnson, 1995). However, the dried lucerne resulted in reduced emissions as the proportion of the dried lucerne increased in the diet.

4.5 Conclusion

The objective of this study was to evaluate either similar enteric methane emissions or reduced emissions between maize silage and dried lucerne diets. The replacement of maize silage with dried lucerne resulted in almost equal absolute enteric methane emissions between maize silage and dried lucerne TMRs. The replacement of maize silage with dried lucerne resulted in reduced enteric methane emission per unit DMI in all the predicted methane productions with increased total methane production as DMI increases. Similarly, the replacement of maize silage with dried lucerne resulted in almost equal enteric methane production for the two TMRs per unit milk yield per day, as lucerne inclusion in TMR was increasing with increasing milk production. This implies that enteric methane production can be reduced as a proportion of dry matter intake when lucerne is included in dairy cows' diet.

Therefore lucerne, or similar crops, have a potential role to play in mitigating enteric methane emission in dairy cows, as well as improving the cows' production efficiency, which results in decreased enteric methane emissions. This should be investigated further, firstly as a desk top study with different protein feeds and different feed qualities. This desk top studies should then be checked with feeding experiments on animals in chambers to accurately measure green house gas emissions.

5. Chapter Five: Experiment 4 Measurement of enteric methane emissions to evaluate the effect of genetic line, dairy cow activity and time of measurement on methane emission of dairy cows offered a high forage total mixed ration (TMR) containing lucerne forage using a laser methane detector

5.1 Introduction

Dairy cows, as ruminant livestock, produce globally about 80Tg of methane annually (Beuchemin *et al.*, 2007). It is reported that in 2007, dairy farming in Scotland contributed 1.5 MTCO₂e, representing 3.0 % of the greenhouse gas (GHG) emissions for the whole of the country (Sheane *et al.*, 2011). Under the 2008 Climate Change Act, the UK aims to reduce greenhouse emissions by 80%, based on the 1990 GHG emissions baseline by 2050 (Lewis, 2008; OPSI, 2008). However, Scotland aims to reduce 42 % and 80 % of its total global methane contribution by 2020 and 2050, respectively (Renwick and Wreford, 2011; Sheane *et al.*, 2011).

In dairy cows' enteric methane abatement, it is important that the enteric methane emissions are accurately measured from the different diets and activities being performed by the dairy cows (Johnson and Johnson, 1995). In dairy cows enteric methane emission, different methods are used to measure methane emission such as respiratory calorimetry chambers, tracer gas techniques and ventilated hood or headbox (Johnston and Johnston, 1995). The respiratory calorimetry chamber measures enteric methane emission with high accuracy but its use is limited by the number of cows to be measured and it is very expensive to construct it (Bhatta *et al.*, 2007).

One method of estimating methane output is to use a laser methane detector (LMD) (Chagunda *et al.*, 2009). LMD technology does not require the animal to be placed in a chamber and can therefore be used for grazing cattle and on commercial farms. Laser methane detector is being validated against standard enteric methane measuring equipment in dairy cows (Chagunda *et al.*, 2010). In the LMD validation study against the respiratory calorimetric chamber by Chagunda *et al.*, (2010), the relationship was strong ($r=0.8$) and

positive with high levels of agreement. In this study, the laser methane detector measurements were validated by the enteric methane prediction published equations to evaluate the strength of the relationship of the two methods of estimating methane.

Laser methane detector measurements are based on infrared absorption spectroscopy principle, using a semiconductor laser beam for methane measurement (Tokyo Gas Engineering, 2006). The concentration of methane gas between the LMD and the target point is measured by the laser beam transmitted towards the target point, in parts per million metres (ppm-m) (Tokyo Gas Engineering, 2006). The equipment was designed to specifically detect methane gas concentration and not other gases such as butane and propylene (Tokyo Gas Engineering, 2006).

The LMD operates fairly well in standard temperatures ranging between 0-40°C, in the absolute humidity range of 20-90%. The laser methane detector measures methane concentration in the range between 10 and 10,000 ppm-m (Tokyo Gas Engineering, 2006) but it is important that for its successful use in dairy farms the measurements from this equipment can be related to other standard established methods for enteric methane estimation, such as methane prediction regression equations (Chagunda *et al.*, 2009). Therefore the LMD and published enteric methane emission predicting equations were used to generate emission data from two different genetic line cows, performing different activities at different time of the day while feeding on high forage TMR containing lucerne. The dairy cows' activities such as ruminating, feeding and resting have either strong positive or negative effect on enteric methane emission (Chagunda *et al.*, 2009).

This study aimed to both determine the relationship between the laser methane detector and methane prediction published equations and factors affecting enteric methane measurements and emission in dairy cows feeding on lucerne based diet.

5.2 Materials and methods

The study was carried out using cows in the Langhill herd at Crichton Royal farm. The Langhill cows are divided into two genetic groups comprising of a select (s) and control line (c) (Veerkamp *et al.*, 1994). The select group (s) has a pedigree of high genetic merit for fat and protein yield with improved milk production and have been bred from UK bulls of known high milk fat plus protein genetic merit from 1973. The control lines (c) are the offspring of the bulls with the national mean genetic merit for fat and protein milk, average milk production inclusive, since 1973 (Veerkamp *et al.*, 1994).

The cows were fed total mixed ration (TMR) containing lucerne, which was provided on *ad libitum* basis. The total mixed ration contained crimped wheat, lucerne, red clover and beans in the ratio of 9.0, 5.0, 23.0 and 13.0 respectively. The cows were under housing system throughout the enteric methane measurement period. Enteric methane measurements were carried out using the laser methane detector (LMD). The methane measurements were taken in the morning (between 10:00 and 12:00) and after mid-day milking (between 14:00 hours and 16.00 hours) and the cows' activities during measurements were recorded. The enteric methane measurements were taken when the cows were feeding in the HOKO gate (Insetec), ruminating or just lying down (idle/resting). The enteric methane measurement was done at a 3m distance to let the cow concentrate on its activity and the measurement was discontinued whenever the cow changed its activity during the 4 minutes measurements. Enteric methane emission measurements were taken from 60 cows selected at random from the group. Five measurements were taken from each cow on five different days and measurements were taken once a day per cow for four minutes for each cow. The measurements were done randomly from the cows and not all of them were measured each day. The measurements were taken by a single operator who stood 3 meters from the cow's head. The animal's activity was also recorded as lying/standing; feeding / ruminating/ drinking/ none. If the animal changed activity within the 4minutes then this recording was discarded. The daily methane production was estimated using the technique

described by Chagunda *et al.* (2009) which takes into account activity and time budgets for an average cow. The LMD measurements were validated by using enteric methane predictions calculated from published equations. Data on DMI were obtained from the Langhill data base for the cows DMI during the emission measuring period.

The amount of feed consumed by each cow was automatically recorded by the HOKO gates (Insetec), as well as time of feeding. The dairy cows' activities at the time of enteric methane measurement were recorded as feeding, rumination and resting/idle. Milk yield was recorded daily from each cow and fat and protein composition was recorded on a weekly basis. Milk yields were converted to energy corrected milk (ECM) yields using the following equation:

$$\text{ECM (kg)} = 0.25 \times \text{M (kg)} + 12.2 \times \text{Fat (kg)} + 7.7 \times \text{Protein (kg)}$$
, where M is the mass of milk, (Sjaunja *et al.*, 1990).

The laser methane detector displays enteric methane measurements in ppm-m but these methane concentrations are the cows' inhaling and exhaling breathing cycle peaks. The standard deviation of each cow's breathing cycle peaks was calculated and any troughs below this line were filtered because they were assumed to be the cows' inhaling troughs. The average of the cows' exhaling peaks was calculated in ppm-m.

The total laser methane detector (LMD) enteric methane measurement was calculated by disregarding the cows' activity during measurement. However, the methane measurements were calculated with reference to the cows' activities to evaluate the effects of the activities on enteric methane emission. The enteric methane measurements taken in the morning before noon were added together and those taken in the afternoon before 16.00hours were also added together to evaluate the effect of time on enteric methane emission regardless of the cows activities.

The cow spends 4 hours on eating, drinking and milking activities and 2 hours on socialising whereas another 14 hours is spent on ruminating (Chagunda *et al.*, 2009). Cooper *et al.*, (2007) reported (in Chagunda *et al.*, 2009) that cows spent 4 hours lying and resting. The cows' daily time budget on different activities is therefore used in calculating mean enteric methane emission but this may change due to the different activities being performed by the cows (Forbes 1995). The LMD measured enteric methane emissions, in ppm-m, were converted to grams per day by using the following equation: $M_{DG} = 0.000576 \times M_{TV} \times TVr$ (Chagunda *et al.*, 2009), where M_{DG} is enteric methane detected by the LMD in grams, M_{TV} is the enteric methane in the breath in ml and TVr is the tidal volume (TVr when the cow is lying down is 3100 ml and when the cow is standing is 3800 ml) (Tenney, 1982). The detailed information on the formulae for converting LMD methane measurements to grams per day is given in appendix 2. The enteric methane emissions were then converted to Mega Joules per day by multiplying by 55.65 MJ (The unit energy mass of methane in kilograms); (Crutzen *et al.*, 1986).

The calculated enteric methane emissions were then converted to both methane emission per unit DMI and milk yield (ECM) in MJ/d/kg DMI and MJ/d/kg milk yield, respectively.

The enteric methane emissions were predicted using the DMI of the cows and the nutrient composition characteristics of the high forage feed containing lucerne forage. The prediction equations used in this study were those developed by Kriss, (1930), Axelsson, (1949), Yan *et al.*, (2005), Yates *et al.*, (2003) and both linear and nonlinear Mills *et al.* (2003) equations based on the diet metabolisable energy intake (MEI) (Table 5-1). These equations were chosen in this study because they are the most frequently used equations in enteric methane studies and their input variables were readily available from the cows' high forage diets.

Table 5-1: Enteric methane prediction equations

Prediction equation	Source
$CH_4 \text{ (MJ/d)} = 1.36 + 1.21 \times DMI - 0.825 \times CDMI + 12.8 \times NDF$	Yates <i>et al.</i> , (2003)
$CH_4 \text{ (MJ/kg)} = 8.25 + 0.07 \times ME \text{ (MJ/Kg)}$	Mills <i>et al.</i> , (2003)
$CH_4 \text{ (MJ/d)} = 45.89 - (45.89 + 0) \times e^{(-0.003 \times MEI \text{ MJ/d})}$	Mills <i>et al.</i> , (2003)
$CH_4 \text{ (MJ/d)} = (47.82 \times DMI \text{ (kg/d)} - 0.762 \times DMI \times DMI - 41) \times 0.03954$	Yan <i>et al.</i> , (2005)
$CH_4 \text{ (MJ/d)} = (75.42 + 94.28 \times DMI \text{ (kg/d)}) \times 0.05524$	Kriss, (1930)
$CH_4 \text{ (MJ/d)} = -2.07 + 2.636 \times DMI \text{ (kg/d)} - 0.105 \times DMI \times DMI \text{ (kg/d)}$	Axelsson, (1949)

The measured enteric methane emissions from the cows and the estimated enteric emissions were examined using ANOVA in SAS system (SAS Inst. Inc., 2001) and regressions and correlation to determine the relationship between the two methods of estimating enteric methane in dairy cows. The ANOVA was used to determine the effect of different factors on enteric methane measurements and emission using linear mixed model with the cow as a random variable as shown in the following statistical model:

Methane (M) $Y_{ijklm} = \mu_{ijklm} + G_i + L_j + A_{Kij} + T_{ln} + \varepsilon_{ijklm}$, where Y= mean enteric methane emission, G= genetic line, L= lactation days, A= cow activity (feeding, ruminating and resting/idle), T= time of enteric methane measurement (morning or afternoon) and ε is the error.

The energy corrected milk yield was adjusted for days in milk because the cows used in this study were in different stages of lactation.

The regression analysis was performed to determine the relationship between the enteric methane emissions predictions of select and control cows from the six enteric methane predictions published equations and those measured using the LMD.

5.3 Results

The enteric methane emission was predicted from the cows whose emission was measured by the LMD using regression equations and the nutrient composition and DMI of the high forage total mixed ration, containing lucerne, on which the cows were feeding. The mean predicted and LMD

calculated/ measured enteric methane emission measurements, the dairy cows DMI and energy corrected milk yield are presented in Table 5-2.

Table 5-2: Mean predicted and LMD enteric methane emission in MJ/d

Prediction Equation	n	Mean	SD	Minimum	Maximum.
Yates et al., (2003) (CH ₄ MJ/d)	60.0	23.2	3.1	15.8	30.3
Mills et al., (2003) (CH ₄ MJ/d)	60.0	21.4	1.9	16.4	25.8
Mills et al., (2003) [nonlinear equation]	60.0	19.5	2.1	14.1	25.0
Axelsson, (1949) (CH ₄ MJ/d)	60.0	11.0	3.0	1.5	14.5
Yan et al, (2005) (CH ₄ MJ/d)	60.0	23.4	2.3	16.2	26.9
Kriss, (1930) (CH ₄ MJ/d)	60.0	167.6	13.2	135.6	198.8
LMD CH ₄ measurements	60.0	29.1	5.6	14.5	39.6

SD=standard deviation, n= number of cows under study

The measured enteric methane emissions were converted to methane per unit DMI and unit energy corrected milk yield respectively to evaluate methane reduction. In dairy cows, as ruminant livestock, enteric methane increases with increasing DMI irrespective of the type of feed (Johnston and Johnston, 1995). The calculated absolute methane emission, methane emission per unit DMI and ECM are presented in Table 5-3.

Table 5-3: Dairy cows DMI (kg/d) and energy corrected milk yield (kg/d)

Dairy cow Select (s)					
	n	Mean	SD	Minimum	Maximum
DMI (kg/d)	25.0	18.6	2.5	12.3	24.8
ECM (kg/d)	25.0	30.8	7.3	17.2	44.6
Dairy cow Control (c)					
	n	Mean	SD	Minimum	Maximum
DMI (kg/d)	35.0	17.0	2.4	11.6	22.7
ECM (kg/d)	35.0	25.3	7.3	14.2	43.6

SD= standard deviation, DMI= Dry matter intake, ECM= Energy corrected milk, n= number of cows

The mean LMD enteric methane emission was $29.10 \pm (SD=5.62)$ MJ/d which ranged from 14.50 to 39.60 MJ/d. The mean DMI for the select cows was $18.6 \pm (SD=2.48)$ kg/d more than the DMI for the control cows $17.0 \pm (SD=1.80)$ kg/d. The total mean energy corrected milk production was high in high genetic select cows, with mean milk production of $30.8 \pm (SD=2.48)$

compared to the $25.3 \pm (\text{SD}=2.43)$ kg/d for the average genetic control line cows.

The mean enteric methane emission predictions in this study ranged from $11.0 \pm (2.95)$ to $167.6 \pm (\text{SD}=13.16)$ MJ/d on average with Kriss (1930) equation predicting the highest and Axelsson (1949) the lowest emission (Table 5-2). The LMD enteric methane measurements were on average $29.1 \pm (\text{SD}=5.62)$ MJ/d.

The results of the regression between the published equations [Yates *et al.* (2003), Mills *et al.*, (2003) linear, Mills *et al.*, (2003) non-linear, Yan *et al.*, (2005), Axelsson (1949) and Kriss (1930)] predicted enteric methane emissions and observed LMD measurements are presented in Table 5-4. The significance of the methane prediction equations regression slope from the line of perfect agreement (LPA) is presented in Table 5-5. The equation of Mills *et al.*, (2003) linear had the highest closer agreement with the LPA compared to the other equations with Kriss (1930) equation having the highest significance. The regressions between the published equations and the LMD enteric methane measurement are graphically presented in Figures 5-1 to 5-6.

Table 5-4: The relationship between published equations and LMD (observed) enteric methane emissions

Equations	Enteric methane estimation (MJ/d)			
	Predicted	Actual (Observed)	Correlation coefficient (r)	RMSPE %
Yates <i>et al.</i> , (2003)	23.2	29.1	0.65, $P<0.001$	2.5
Mills <i>et al.</i> , (2003) linear	21.4	29.1	0.65, $P<0.001$	2.6
Mills <i>et al.</i> , (2003) [nonlinear equation]	19.5	29.1	0.66, $P<0.001$	2.9
Yan <i>et al.</i> , (2005)	23.4	29.1	0.57, $P<0.001$	2.5
Kriss (1930)	167.6	29.1	0.65, $P<0.001$	34.7
Axelsson (1949)	11.00	29.1	0.54, $P<0.001$	6.7

Table 5-5: The significance of the predicted equations regression slope from the line of perfect agreement (LPA)

Equation	Calculated t value	Critical t values at 95% CI	Degrees of freedom (V)
Yates <i>et al.</i> , (2003)	1.48	2.92	2
Mills <i>et al.</i> , (2003) linear	2.89	2.92	2
Mills <i>et al.</i> , (2003) non linear	2.50	2.92	2
Yan <i>et al.</i> , (2005)	2.43	2.92	2
Kriss (1930)	-0.28	2.92	2
Axelsson (1949)	1.73	2.92	2

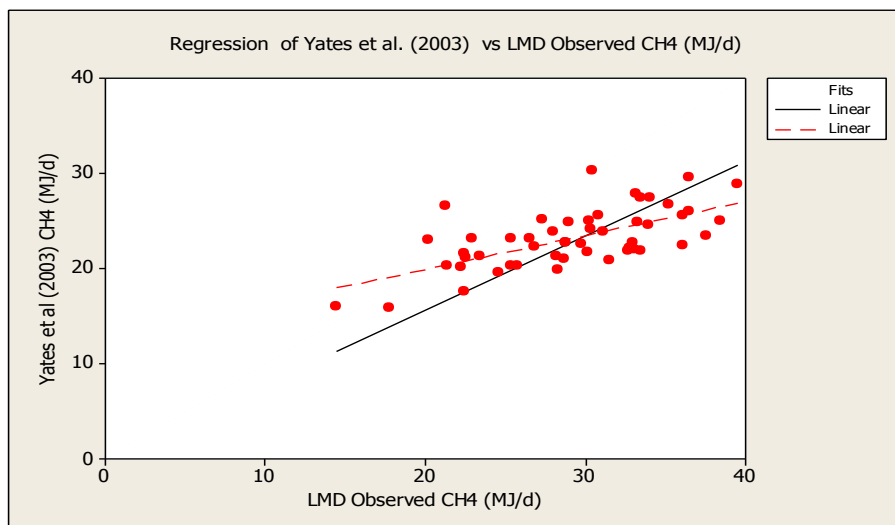


Figure 5-1: The regression and correlation between the LMD CH₄ measurements and prediction by Yates *et al.*, (2003) equation, Yates *et al.*, (2003) CH₄= 12.8 + 0.356 LMD CH₄ (MJ/d)

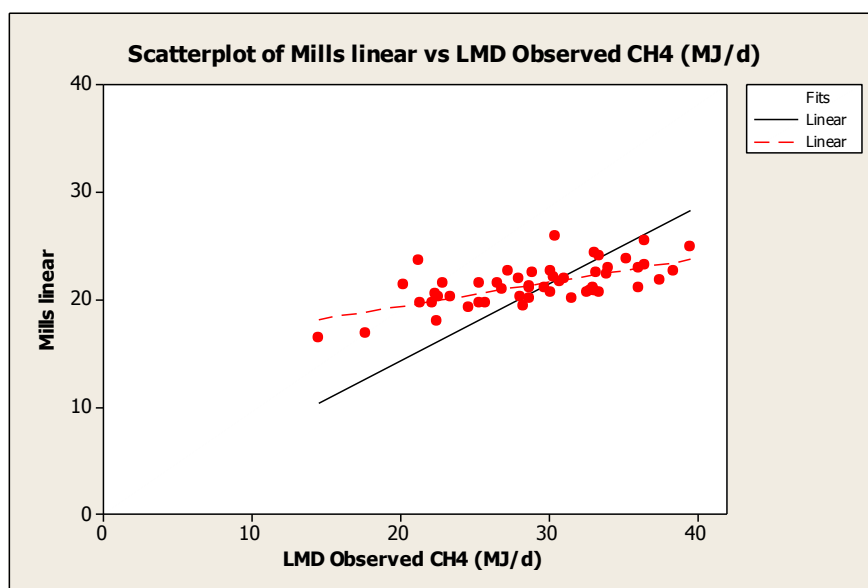


Figure 5-2: The regression and correlation between the LMD CH₄ measurements and prediction by Mills *et al.*, (2003) equation, Mills *et al.*, (2003) CH₄ (MJ/d)= 14.9 + 0.222 LMD CH₄ (MJ/d)

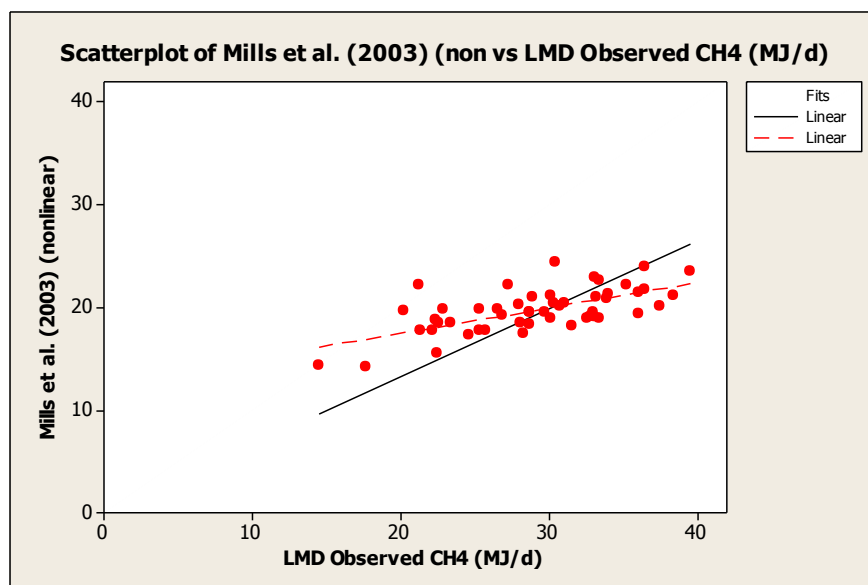


Figure 5-3: The regression and correlation between the LMD CH₄ measurements and prediction by Mills *et al.* (2003) nonlinear equation, Mills *et al.* (2003) = 12.5 + 0.247 LMD CH₄ (MJ/d)

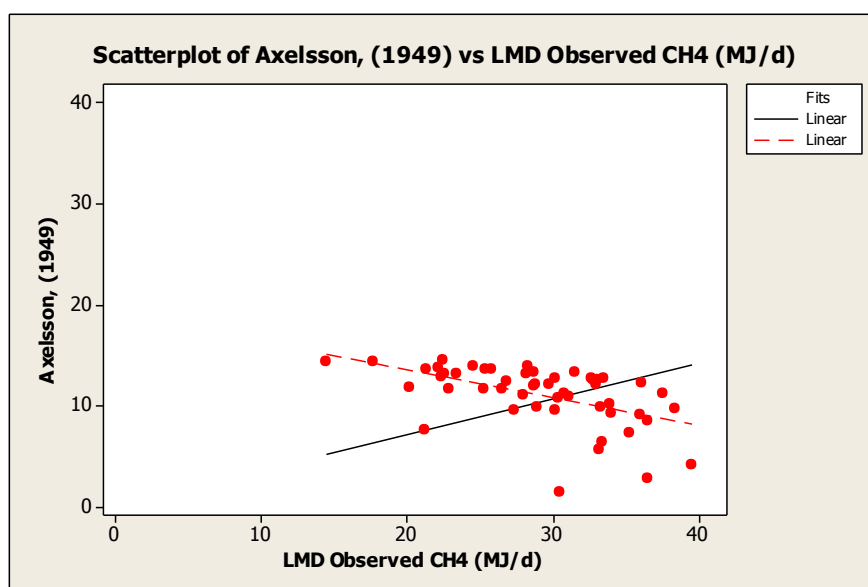


Figure 5-4: The regression and correlation between the LMD CH₄ measurement and prediction by Axelsson (1949) equation, Axelsson (1949) CH₄ (MJ/d) = 19.2 + 0.280 LMD CH₄ (MJ/d)

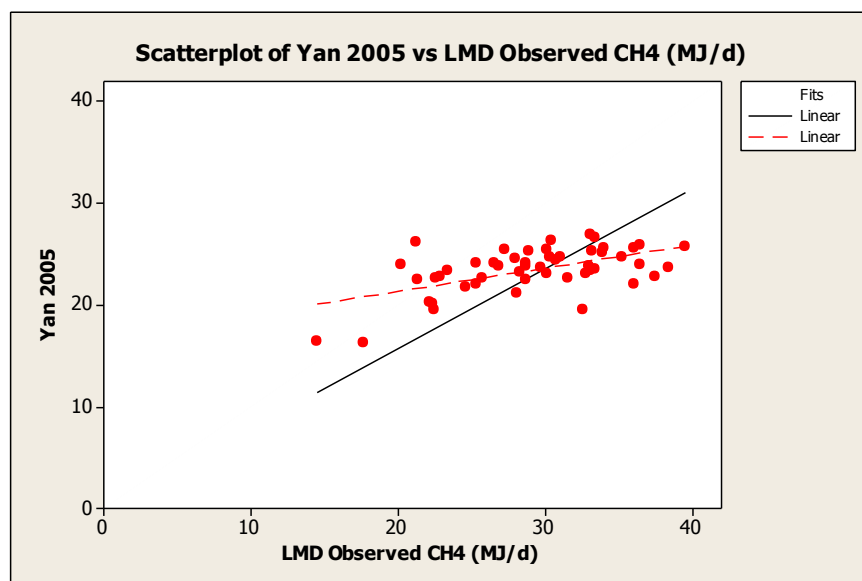


Figure 5-5: The regression and correlation between the LMD CH₄ measurements and prediction by Yan *et al.*, (2005) equation, Yan *et al.* (2005) CH₄ (MJ/d) = 16.8 + 0.226 LMD CH₄ (MJ/d)

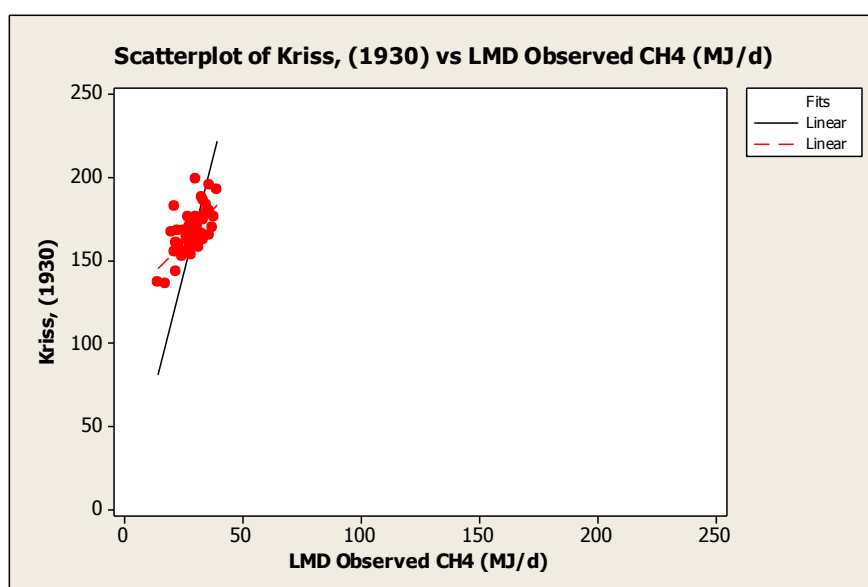


Figure 5-6: The regression and correlation between the LMD CH₄ measurements and prediction by Kriss (1930) equation, Kriss (1930) CH₄ (MJ/d) = 123 + 1.52 LMD CH₄ (MJ/d)

The activity of the cows had an effect on the amount of enteric methane emission and measurements (Table 5-6). The cows that were ruminating in the HOKO gates (Insetec) emitted more ($P<0.05$) enteric methane than those which were either idle/resting or feeding. The cows which were feeding in the HOKO gates (Insetec) produced more ($P<0.05$) enteric methane than the cows which were idle/resting (standing and lying). The mean enteric methane emission for the cows that were ruminating was $21.5 \pm (SE=9.7)$ MJ/d whereas those which were feeding produced $18.6 \pm (SE=9.7)$ MJ/d representing a 15.6% enteric methane emission increase (Table 5-6). The cows which were idle / resting (standing/lying) produced less ($P<0.05$) enteric methane emission of $13.0 \pm (SE=7.9)$ MJ/d than ruminating and feeding cows representing an enteric methane emission decrease of 39.5 and 30.1%, respectively. In general, ruminating cows, including those which were feeding, produced more ($P<0.05$) enteric methane than those which were idle/ resting. This means that irrespective of the cow's other activities, rumination had a great influence / effect on enteric methane emission.

Table 5-6: Select and Control cows' enteric CH₄ emission per day, ECM and DMI in MJ/d

Factor	Level	CH ₄ (MJ/d)		CH ₄ /ECM (MJ/d)		CH ₄ /DMI (MJ/d)	
		LSM	SE	LSM	SE	LSM	SE
Cow activity							
	idle/resting	13.0 ^a	7.9	0.42 ^{aa}	0.07	1.01 ^{aaa}	0.5
	Feeding	18.6 ^b	9.7	0.45 ^{bb}	0.07	1.05 ^{bbb}	0.5
	Ruminating	21.5 ^c	9.7	0.46 ^{cc}	0.07	1.17 ^{ccc}	0.5
Time of measurements							
	Morning	16.7 ^d	7.9	0.45 ^{dd}	0.07	1.14 ^{ddd}	0.5
	Afternoon	18.5 ^e	9.7	0.43 ^{ee}	0.07	1.02 ^{eee}	0.5
Cow genetic line							
	Select	19.9 ^f	9.7	0.50 ^{ff}	0.07	1.10 ^{fff}	0.5
	Control	19.2 ^g	9.7	0.60 ^{gg}	0.07	1.10 ^{fff}	0.5

DMI= dry matter intake, SE= standard error, LSM= Least Square mean, ECM= Energy corrected milk, means with different superscripts in each column are significant ($P<0.05$), n=60

The time of enteric methane measurement had an influence / effect on the enteric methane emission. The dairy cows produced more ($P=0.04$) enteric methane in the afternoon after milking when they were feeding in the HOKO gates (Insetec) than in the morning (Table 5-6). The total mean enteric methane emissions produced by the cows in the afternoon between mid-day and 16.00 hours and in the morning from 10.00 hours to 12.00 hours was $18.5 \pm (SE=9.7)$ and $16.7 \pm (SE=7.9)$ MJ/d, representing a difference of 10.8 % enteric methane emission increase in the afternoon.

In this study the LMD was proven to have strong correlation with the enteric methane production prediction statistical equations. The validated measurements from the LMD were therefore used to verify the Langhill cows into their groups with reference to the enteric methane produced as the cows were feeding on the lucerne based diet (Table 5-6).

The Select cows (S) produced higher ($P<0.05$) mean enteric methane of $19.9 \pm (SE=9.7)$ MJ/d whereas the Control cows' (C) mean enteric methane emission was $19.2 \pm (SE=9.7)$ MJ/d, representing an enteric methane emission difference of 3.5%. The mean enteric methane emission per unit DMI was equal ($P<0.05$) in both select (S) and average genetic control (C) cows with a mean emission of $1.1 \pm (SE=0.5)$ MJ/d. In high genetic select line cows, the enteric methane emission per unit energy corrected milk production was lower ($P<0.01$) than that of the average genetic control cows. The high genetic select cows produced lower ($P<0.01$) enteric methane of $0.5 \pm (SE=0.07)$ MJ/d/ milk yield enteric methane whereas the average control cows produced $0.6 \pm (SE= 0.07)$ MJ/d/ milk yield enteric methane.

5.4 Discussion

The enteric methane measurements from the LMD had a strong correlation with Yates *et al.* (2003) equation but were slightly underestimated compared to the LMD methane measurements. Yates *et al.* (2003) equation underestimated enteric methane emission by 20.3% units compared to the mean LMD emission measured value. Bell *et al.* (2009) estimated the mean

enteric methane emission at $25.7 \pm (\text{SD}=3.6)$ MJ/d whereas the LMD and Yates *et al.* (2003) methane measurements and prediction in this study had a mean methane emission value of $29.1 \pm (\text{SD}=4.35)$ MJ/d and $23.2 \pm (\text{SD}=3.08)$ MJ/d respectively. The high LMD enteric methane measurements could be attributed to the high concentration of methane which would have been emitted by the burping cows during enteric methane measurement. The strong correlation coefficient ($r = 0.65$) with $r^2 = 0.42$, between predicted methane emission from Yates *et al.* (2003) and the LMD measurements means the regression equation from this relationship can be used to adjust for the errors occurring in the LMD when measuring enteric methane emission in Langhill cows. This suggests that about 42.8% of the variations in Yates *et al.* (2003) equation ability to predict methane emission can be explained by the ability of the laser methane detector. This means the two methods of estimating enteric methane in dairy cows are strongly dependent on one another in such a way that a change in LMD measurements affects Yates *et al.* (2003) methane estimation. The small regression error/root mean square prediction error (RMSPE) (2.5% of the LMD mean) indicates that the Yates *et al.* (2003) equation predicts enteric methane with high accuracy with reference to the LMD measurements although some improvements in some factors in both the LMD and the Yates *et al.* (2003) equation are needed (Wilkerson *et al.*, 1995; Ellis *et al.*, 2007). However, the small slope between this equation and the line of perfect agreement (LPA) (Table 5-5) suggested that the accuracy was less than that of Mills *et al.* (2003) linear equation.

The published Mills *et al.* (2003) linear equation predicted mean enteric methane emissions had a strong correlation with the observed LMD methane measurements. Mills *et al.* (2003) linear equation under-predicted the mean enteric methane emissions on average by 26.6% compared to the LMD mean value. The strong correlation suggests that there are small variations in the enteric methane emission estimations in the Langhill cows between the linear equation of Mills *et al.* (2003) and the LMD. This means these two methods of estimating enteric methane emission in dairy cows are dependent of one another and a change in the values of one equation

affects the other. The mean enteric methane emission of the Langhill cows predicted by Bell *et al.* (2009), using the linear equation of Mills *et al.* (2003), was $24.0 \pm (\text{SD}=3.5)$ MJ/d whereas the predicted and measured emissions in this study were $21.4 \pm (\text{SD}=1.90)$ and $29.1 \pm (\text{SD}=5.62)$ MJ/d respectively. The LMD had high enteric methane measurements compared to the two estimates which could be attributed to the ruminal methane when the cow was burping. The small root mean square prediction error/RMSPE (2.6% of LMD mean) indicates that the Mills *et al.* (2003) linear equation predicts the enteric methane emissions in Langhill cows with high accuracy although improvements are required in some factors in both the LMD and the equation (Wilkerson *et al.*, 1995; Ellis *et al.*, 2007). Mills *et al.* (2003) linear equations prediction accuracy is lower than that of Yates *et al.* (2003) equation. However, the slope between the Mills *et al.* (2003) linear equation and the LPA suggested that this equation has the highest prediction accuracy with respect to the LMD enteric methane measurement.

The nonlinear regression equation of Mills *et al.* (2003) predicted mean enteric methane emissions were underestimated by 33.0% compared to the LMD mean methane measurements. Chagunda *et al.* (2009) measured mean methane emissions using the LMD and found a mean value of 24.46 MJ/d. The predicted enteric methane emissions in this relationship had a strong correlation coefficient ($r^2 = 0.43$) with the laser methane detector methane measurements, indicating that the regression equation between the two can be used to offset some errors in the methane predictions (Wilkerson *et al.*, 1995). This suggests that about 43% of the variations in estimating enteric methane emissions by the nonlinear equation of Mills *et al.* (2003) can be explained by the LMD and therefore are dependent of one another. The small regression error/RMSPE (2.9% of LMD mean) indicates that the nonlinear equation of Mills *et al.* (2003) equation estimates enteric methane emission in dairy cows with accuracy with reference to the laser methane detector's ability in measuring methane emissions (Wilkerson *et al.*, 1995) but the accuracy is less than that of Yates *et al.* (2003) and Mills *et al.*, (2003) equations. However, slope between the Mills *et al.* (2003) nonlinear

equation and the LPA indicated that this equation had better prediction accuracy than Yates *et al.* (2003) equation.

Yan *et al.* (2005) predicted mean enteric methane emissions were underestimated by 19.6% compared to the mean measured emission values from the LMD. The strong correlation coefficient, $r = 0.57$ between Yan *et al.* (2005) equation predicted enteric methane emissions and the measured LMD methane emissions indicates that the regression equation from this relationship can be used to adjust for the differences which affect enteric methane estimation when both the LMD and Yan *et al.* (2005) equations are used (Wilkerson *et al.*, 1995). The strong correlation coefficient $r = 0.57$ with $r^2 = 0.32$ means that 32% of the variations in methane emission estimation by the Yan *et al.* (2005) equation can be explained by the LMD ability in measuring enteric methane. The small RMSPE in this relationship (2.5% of the LMD mean) indicates that the Yan *et al.* (2005) equation predicts enteric methane emission with accuracy compared to the LMD methane measurements (Wilkerson *et al.*, 1995; Ellis *et al.*, 2007). Yan *et al.* (2005) equation was more accurate in predicting enteric methane emission than the equations of Mills *et al.* (2003) [linear and nonlinear], Axelsson (1949) and Kriss (1930) but similar to that of Yates *et al.* (2003). However, comparing the slope of the Yan *et al.* (2005) equation and the LPA indicates that Yan *et al.* (2005) equation has high prediction accuracy compared to Axelsson (1949), Kriss (1930) and Yates *et al.* (2003) equations.

The Axelsson (1949) and Kriss (1930) equations underestimated and overestimated enteric methane emission by 62.2 %, whereas Kriss (1930) mean enteric methane emission was almost six times overestimated compared to the LMD mean enteric methane emission measurements. The Axelsson (1949) equation's enteric methane emission correlation with the LMD measurements was strong and negative with a correlation coefficient r of 0.54 ($r^2=0.29$). This suggests that 29 % of the variation in the Axelsson, (1949) equation's enteric methane estimates could be explained by the variations in the LMD methane measurements. The RMSPE as a proportion of the LMD mean value in this relationship was 6.2 %, indicating that this

equation estimates enteric methane emission with larger errors compared with the LMD and there is need for improvements in some factors in either the LMD or the Axelsson equation itself (Wilkerson *et al.*, 1995). The accuracy in estimating the enteric methane emission was better than that of Kriss (1930) but poor compared to the other equations. Similarly, comparing the slope of the Axelsson (1949) and the LPA suggested that the prediction accuracy was better than Kriss (1930) but poor with reference to the other equations.

Kriss (1930) predicted methane emissions had a strong correlation coefficient r of 0.65 ($r^2=0.42$) with the LMD enteric methane emission measurements, despite overestimating the enteric methane emissions. This suggests that 42 % of the variations in the ability of the Kriss (1930) equations to predict enteric methane emissions can be explained by the LMD methane measurements. The RMSPE as a proportion of the LMD mean emission was 34.7 % indicating that Kriss (1930) equation predicts methane emission with large errors when compared to the LMD methane emission measurements and therefore Kriss (1930) equation could not be used for methane emission prediction in the Langhill cows (Wilkerson *et al.*, 1995). This means there is need for some improvements to be investigated in estimating enteric methane emission using these two methods (Wilkerson *et al.*, 1995).

In this study, in summary, the predicted enteric methane measurements from the LMD had a strong correlation with the regression equations, indicating that the ability of the LMD to measure enteric methane is similar to that of the regression equations.

In this study, the enteric methane emissions from the dairy cows were higher when the cows were ruminating than when the cows were lying down. The mean enteric methane emission when the cows were feeding in this study was $18.6 \pm (SE=9.7)$ MJ/d whereas Chagunda *et al.* (2009), using the LMD, reported a mean enteric methane emission in feeding dairy cows of $31.94 \pm (SE =2.33)$ MJ/d, representing an 41.8 % enteric methane reduction. The

use of LMD is a relatively new technique and these differences may be due to the procedures followed not being exactly the same as Chagunda *et al.* (2009) for example the exact distance measurements were taken from the cow's head. The high concentration of methane emissions when the cows were feeding, in this study, could be because of the direct emission of methane from the rumen when the cows were feeding. The mean enteric methane emission for the cows that were just standing and lying idly/resting was $13.0 \pm (SE=7.9)$ MJ/d whereas Chagunda *et al.* (2009) reported the enteric methane mean value of $24.7 \pm (SE= 2.31)$ MJ/d for the standing cows, representing 47.4 % reduction in methane emissions. The cows that were ruminating in this study produced $21.5 \pm (SE=9.7)$ MJ/d enteric methane. Chagunda *et al.* (2009) reported a mean enteric methane emission value of $27.7 \pm (SE= 0.97)$ MJ/d for ruminating cows representing 28.8% increase in enteric methane emission. Marik and Levin, (1982) reported (in Chagunda *et al.*, 2009) that during ruminating, dairy cows produce more enteric methane than any other time activity because the movements of the upper part of the digestive tract result in the release of high concentrations of methane. The difference in enteric methane emissions between this study and that of Chagunda *et al.* (2009) could be attributed to the difference in the nutrient content of the diet the cows were being fed, as the cows in this study were feeding on high forage diet containing lucerne. In Chagunda *et al.* (2009) the cows were fed on both high and low forage feed but when forage was insufficient, feed comprising between 0.30 and 0.25 concentrate supplement of the total diet was used. The high differences in the enteric methane emission between the two studies could also be attributed to personal errors in measuring and calculating the emission using the laser methane detector equipment. Furthermore dairy cows as biological animals could also be the source of the differences in enteric methane because biologically ruminal digestion and fermentation of the diet may also change with time and is not constant.

The enteric methane production from the dairy cows was higher in the afternoon after milking than it was in the morning, irrespective of the cows' behaviour / activity. This could be because the cows were standing as they

were feeding in the HOKO gates (Insetec) unlike in the morning, when the cows were lying idly.

One objective of this work was to identify the two genetic lines of the Langhill cows (control and select) from the validated LMD enteric methane measurements produced from the cows as a result of feeding on the high forage feed containing lucerne. Dairy cows with improved genetic merit for high milk production have higher dry matter intake (DMI) than low genetic merit dairy cows for milk production (Kirchgessener *et al.*, 1995; O'Mara, 2004). Increasing feed DMI in dairy cows increases enteric methane emission /production irrespective of feed type (O'Mara, 2004; Johnson *et al.*, 1996). Therefore, the Langhill select line cows have an improved milk yield (Chagunda *et al.*, 2009), increased DMI, and it is expected that they will have high absolute enteric methane emission.

The high genetic select line cows had both higher ($P<0.05$) dry matter intake and enteric methane emission production than the average genetic control lines, reflecting well the fact that enteric methane emission is positively correlated with an increase in DMI (Kebreab *et al.*, 2006). It is reported that the genetic select line of the Langhill cows have higher DMI than the average genetic control line (Chagunda *et al.*, 2009). Enteric methane production in dairy cows is positively correlated with increasing DMI of the feed (Johnson and Johnson, 1995; Blaxter and Clapperton, 1965). The improvement in the genetic line of dairy cows improves DMI (Kirchgessner *et al.*, 1995), which results in high enteric methane production. The select and average genetic control line cows had equal enteric methane emission per unit DMI because the average genetic control line cows had lower DMI, resulting in high emission per unit DMI than the high genetic select cows (Chagunda *et al.*, 2009; Kirchgessner *et al.*, 1995; O'Mara, 2004; Blaxter and Clapperton, 1965; Johnson and Johnson, 1995). However, despite the high enteric methane emission in the high genetic select line cows, the enteric methane emission per unit milk yield in genetic select cows was lower than that of the average genetic control line cows, agreeing well with the fact that

improved genetic select cows have low enteric methane emissions because of the high milk production.

5.5 Conclusions

The study showed that in estimating enteric methane, there is a strong relationship between the laser methane detector (LMD) and some of the published regression equations used in this study. This study demonstrated that the cow's activity had strong influence on the enteric methane emission and measurements when the cows were feeding on the feed containing lucerne forage. This study, further demonstrated that the dairy cows' genetic line has an influence on the enteric methane emission of the Langhill cows when they are feeding on lucerne based diet. This study demonstrated that genetic selection of dairy cows with both reduced DMI and enteric methane production per unit DMI has a potential in mitigating enteric methane emission as well as increasing milk production.

In this study there was no emission data from grass based total mixed ration to compare with the enteric methane emission levels from the Langhill cows feeding on the TMR containing dried lucerne. However, it is recommended that the enteric methane emissions of cows fed on lucerne and grass based diets should be measured using the laser methane detector to compare the different enteric methane produced with reference to dairy cow activity, genetic line and emission reduction. This would be relevant for smallholder dairy farms in the developing world where dairy cows are fed on more grass than cereals, such as Malawi.

6. Chapter Six: General Discussion

The competition for food between man and dairy cows, coupled with the universally increasing demand for milk consumption and production, requires that cheap but high nutritious forages are used to feed dairy cows. Lucerne, as a legume forage, grows well with higher yields than weeds in summer. However, lucerne becomes dormant in winter and therefore the presence of weeds in the field reduces its yield at harvesting time because of competition for nutrients and sunlight. In winter, lucerne yield is adversely affected by the frost because lucerne does not compete well with weeds for food, resulting in a higher yield of weeds than lucerne.

The advent of climate change poses great challenges to dairy farmers in many regions to grow drought resistant forage crops to sustain their business. However, because lucerne has deep tap roots, it is capable of growing in low rainfall areas and does well with irrigation. Lucerne grazing at both the winter onset and before mid-winter has no effect on spring lucerne yield. This means that because lucerne is dormant during winter, it can be either harvested or grazed at the beginning of winter to feed dairy cows when grass is insufficient. This shows that lucerne can sustain dairy production throughout the year without experiencing any feed shortage during winter. Lucerne, being leguminous forage, is capable of fixing nitrogen in the soil and therefore can be cultivated with no inorganic fertilizer application. This further makes the cost of growing lucerne lower, as well as it having fewer adverse environmental effects in terms of carbon pollution than other commonly grown forage crops. The cost of production in terms of cost per tonne is greatly influenced by yield per ha and the studies reported in this thesis show that weed infestation can be a problem for winter lucerne cultivation. More detailed experimental work is required to evaluate the effect of different weed infestation proportions on yield, cost of production, nutritive quality and efficacy of ensiling.

It is well documented that lucerne is hard to ensile, resulting in many farmers not opting to grow it. The ensiling of lucerne is greatly affected by cold

weather because of prolonged respiration after harvesting the forages (Phipps *et al.*, 1997). The prolonged respiration results in reduced fermentation substrates, such as water soluble carbohydrates (WSC) for lactic acid bacteria. Drier weather adversely affects the lucerne ensiling process by reducing WSCs through the loss of a high proportion of leaves, as the leaves contain more nutrients than stems (Lancefield *et al.*, 2009). This means that lucerne is an ideal forage crop for the mitigation of climate change in Scotland, as it is expected that climate change may bring warmer weather. However, for sub-Saharan African countries like Malawi, the expected warm weather may be more suitable for ensiling lucerne than the current drier conditions. The ensiling of lucerne with biological inoculants such as *Lactobacillus plantarum* MTD-1 results in well fermented, good quality silage. The application of *Lactobacillus plantarum* MTD-1 in lucerne silage ensiling produces quality silage with an improved nutrient content and aerobic stability when the silos are opened.

The use of low quality forage crops in dairy cows' total mixed ration reduces milk production while increasing environmental pollution because of high losses of dietary energy during ruminal fermentation in the form of enteric methane. The inclusion of lucerne in dairy cows' total mixed rations reduces the amount of dietary energy lost, in the form of enteric methane, and improves milk production as it would have been when the dairy cows were fed on maize silage. Therefore, lucerne can be used in dairy farming to improve milk production with no detrimental effects on the environment.

The laser methane detector and published enteric methane estimation equations have shown to measure enteric methane production in dairy cows feeding on a lucerne diet with some degree of correlation. Enteric methane measurements using both the LMD and published equations strongly depend on the dairy cows' activity, genetic line, and time of measuring as well as the type of feed being offered. Enteric methane measurement and estimation using both the LMD and published equations should be conducted on grass based feed in order to compare the reduction of methane production with that of a lucerne based diet.

Lucerne, therefore, can be used in developing countries like Malawi, to improve dairy cow farming among poor smallholder dairy farmers who cannot afford to both buy and cultivate enough maize grain to feed the dairy cows for improved milk production. In developing countries such as Malawi, milk consumption is about 5-6 times lower than in developed countries because of low production (Delgado *et al.*, 1998). In Malawi, smallholder dairy farmers produce three fifths of the total milk production, with two fifths being produced by large scale farms (Chagunda *et al.*, 2010b). There are about 3600 smallholder dairy farmers, with about five large scale farms (Imani Development Consultant, 2004), who produce 35,000 metric tonnes of milk annually (Chagunda *et al.*, 2006; Iman Development Consultant, 2004). This level of milk production is lower than the milk production in other developing countries and, as a result, milk consumption per capita in Malawi is between 4.5 and 6.0 kg compared to the recommended consumption rate of 15 kg and 200 kg for African countries and FAO respectively (Chagunda *et al.*, 2010b). It is therefore recommended that this study be carried out on a large scale among smallholder farmers in Malawi to improve milk production.

In developed countries, such as Scotland, lucerne feeding in dairy cows would be necessary by 2020 because it is reported that the world human population is expected to increase to about 9.0 billion (Ladosi, 2008; Buttriss, 2011), with a subsequent increase in demand of milk consumption (Delgado *et al.*, 1998; Buttriss, 2011). The increase in milk production may increase the prices of cereals because cereals are expected to become scarce (Delgado *et al.*, 1999). The scarcity and high prices of the cereals may universally make it hard for farmers to feed their animals, especially in developing world.

It is important that this study should be further conducted in the future for a long period of time in both Scotland and Malawi, in order to establish the degree of the adverse effects of both poor weeding and grazing management on lucerne yields and growth rate in spring. It is required that lucerne should be grown in both pure and mixed stands to evaluate the difference in the yield harvested in each stand. The growing behaviour of

lucerne in summer, autumn and spring should be studied in detail to understand how its nutrients are utilised in these seasons, winter season inclusive, for proper grazing management, in order to enhance the lucerne stand's longevity. The lucerne ensiling process as well as duration need to be further studied to establish the cause of reduced lactic acid and increased propionic and acetic acid concentration in *Lactobacillus plantarum* MTD-1 inoculation. The levels of lucerne inclusion in the dairy cows' TMR is of paramount importance for understanding / knowledge of how to both improve milk production and reduce enteric methane emissions without affecting the animals' health. Therefore, nutrient compositions of lucerne at different stages of growth may be developed in the future, by using near infrared reflectance spectroscopy (NIRS) to develop NIRS equations for farmers' use when formulating dairy cow TMRs with DMI, nutrient content, milk yield and enteric methane emissions as input and output variables. This may require continual research on lucerne nutrient quality wet chemistry analyses, near infrared reflectance spectroscopy analyses and in vivo nutrient digestibility in dairy cows. These nutrient analyses should either be equated or regressed in order to develop lucerne forage NIRS nutrient prediction equations. The use of lucerne nutrient NIRS equations may improve the efficiency of using lucerne forages, which could result in improving both milk production and dairy cows' health, as well as reducing enteric methane emission.

7. Chapter Seven: General conclusions

This study has proven that the growth of lucerne could not be adversely affected by poor weeding management as a result lucerne can be grown in mixed stands with other forage crops and lucerne grows well in Scotland in summer. However, because lucerne becomes dormant in winter, its growth is adversely affected by weed infestation and the weeds are advantaged in using nutrients resulting in lower lucerne yield than weeds. The study has demonstrated that lucerne is a high yield crop compared to the weeds which were growing together with lucerne. It is advisable that lucerne should be either grazed or harvested at the onset of winter but before mid-winter in order to harvest high lucerne yield in spring.

After harvesting, lucerne should be wilted to either 280 g/kg DM or above 300 g/kg DM in order to end up with good quality silage after ensiling using *Lactobacillus plantarum* MTD-1 inoculant / additive. *Lactobacillus plantarum* MTD-1 additive has proven to enhance the degree of fermentation, in lucerne forage ensiled on laboratory scale at dry matter content below 300 g/kg DM, on the lucerne nutrient composition, fermentation characteristics and improved aerobic stability after the silages have been opened and exposed to air.

In dairy farming, more dietary energy is lost when grass forages are being offered as animal feed than legumes forages and cereals. This dietary energy loss in livestock animals such as dairy cows is lost in the form of methane. The dietary energy loss, in the form of methane results in low milk production, because of low energetic feed efficiency utilisation and increased global warming resulting in climate change. However, the inclusion of lucerne as forage crop in dairy cows total mixed ration has proven to produce equal absolute enteric methane with maize silage but less enteric methane per unit dry matter intake. Similarly equal concentration of enteric methane has been estimated by dried lucerne with maize silage. Maize silage is a high quality feed and has been used in mitigating dietary energy loss in the form of enteric methane. This means lucerne has a potential in

mitigating enteric methane emission in dairy farming as both a loss of dietary energy, resulting in high milk production and a source of methane resulting in less environmental pollution.

In dairy farming, it is important that enteric methane emission is accurately measured from both different genotype of the cows and the activities performed by the cows without disturbing them. It is important to estimate the effect of the time of the day on enteric methane emission in dairy cows offered different types of diets. In this study it has been proven that the laser methane detector can be used to measure enteric methane while the cows are feeding in their environment. However, the accuracy of measurement using this equipment needs to be improved. The study has further proven that the cows activities such as feeding, resting and ruminating have strong effect on enteric methane emission with ruminating cows emitting more methane than feeding and resting cows. The time of the day has also an effect on enteric methane emission and the cow genotype influence enteric methane emission with select (S) genetic line dairy cows producing more absolute enteric methane than control (C) genetic line cows. However, select genetic line cows have proven to produce equal enteric methane per unit dry matter intake with control cows but less emission per unit energy corrected milk yield than control cows. This means select (S) cows are more efficient in mitigating dietary energy loss in the form of methane than control (C) cows.

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Appendices

Appendix 1 Laboratory chemical analyses

Laboratory chemical analyses procedures for lucerne forage and silage nutrient content analyses are as follows:

1: Dry matter (DM) analysis was done by drying the samples at 100° C for 5 hours to constant weight.

2: Crude protein was done by using Kjeldahl sulphuric acid digestion, analysis by steam distillation.

3: Ash / mineral content was analysed by ashing the sample in muffle furnace at 500° C for 2 hours to constant (MAFF/ADAS RB427).

4: Ether Extract was analysed by extraction in diethyl ether and the residues was hydrolysed with hydrochloric acid and re-extracted with petroleum ether (AOAC Official Method, 2003.5).

5: Neutral detergent fibre (NDF) was analysed by using Foss FibreCap System (Weende and Van Soest methods).

6: Water soluble carbohydrates (WSC) was analysed by using spectrophotometer (Analysis of Agricultural materials RB 427 (MAFF)).

7. pH was analysed by using MAFF / ADAS RB427.33 method.

8: Ammonia-Nitrogen was analysed by MAFF / ADAS RB427.54 method.

Appendix 2 Diet calculations for Experiment 3

Diet: Low dried lucerne no maize silage

Animal Details

Weight:	600 kg
Dairy cow weight change:	0.00kg/day
Milk yield:	20 kg/day
Milk fat:	39 g/day
Milk protein:	34 kg/day
Weeks before calving:	40,
Weeks into lactation	12
Condition score:	2.5,
Lactation no.:	2

Recommended Diet

Feed	Fresh Weight (kg/d)	Dry Weight (kg/d)
Silage average	43.8	10.5
Dairy 13.0 ME 18% protein	1.0	0.87
Dried lucerne	6.0	5.4
Totals	50.8	16.8

Diet cost£ 1.26 per day Cost/litre 6.3 p

Diet intake is 115.6% of maximum predicted intake dry matter

Diet Composition (g/kg DM)

	Whole Diet	Concentrate Only
DM (g/kg)	330	
ME (MJ/kg DM)	10.1	13.0
Crude protein	163	210
Oil	41.0	
NDF	453	
Starch	9.9	
Sugar	39.2	
Long Roughage (%)	94.8	
DCAB (meq/kg DM)	546	
DUP (%MP requirement)	57.2	

Nutrient Balance (g/day)

	Animal requires	Diet supplies	Mineral Deficit
ME (MJ/day)	170	170	
ERDP	1302	1708	
DUP	599	818	
RSV	103	255	

These feeding recommendations are produced as a guide only and do not constitute a guarantee that the predicted levels of performance will be achieved since many other factors have an effect on animal performance. In no event will SAC or the company using this program be liable for damages arising out of the use or misuse of this software.

Diet: Medium dried lucerne no maize silage**Animal Details**

Dairy cow weight:	600 kg
Dairy cow weight change:	0.00kg/day
Milk yield:	25 kg/day
Milk fat:	39 g/day
Milk protein:	34 kg/day
Weeks before calving:	40
Weeks into lactation:	12
Condition score:	2.5
Lactation no.:	2

Recommended Diet

Feed	Fresh Weight (kg/d)	Dry Weight (kg/d)
Silage average:	49.0	11.8
Dairy 13.0 ME 18% protein:	1.5	1.3
Dried lucerne:	7.0	6.3
Totals:	57.5	19.4

Diet cost£ 1.44 per day Cost/litre 5.7 p

Diet intake is 125.8% of maximum predicted intake dry matter

Diet Composition (g/kg DM)

	Whole Diet	Concentrate Only
DM (g/kg):	337	
ME (MJ/kg DM):	10.2	13.0
Crude protein:	164	210
Oil:	41.2	
NDF:	450	
Starch:	12.8	
Sugar:	40.3	
Long Roughage (%):	93.3	
DCAB (meq/kg DM):	541	
DUP (%MP requirement):	58.7	

Nutrient Balance (g/day)

	Animal requires	Diet supplies	Mineral Deficit
ME (MJ/day)	197	197	
ERDP	1507	1943	
DUP	719	985	
RSV	108	252	

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Diet: High died lucerne no maize silage**Animal Details**

Dairy cow weight:	600 kg animal
Dairy cow weight change:	0.00kg/day
Milk yield:	30 kg/day
Milk fat:	39 g/day
Milk protein:	34 kg/day
Weeks before calving:	40
Weeks into lactation	12
Condition score:	2.5
Lactation no.:	2

Recommended Diet

Feed	Fresh Weight (kg/d)	Dry Weight (kg/d)
Silage average	48.0	11.5
Dairy 13.0 ME 18% protein	2.0	1.7
Dried lucerne	10.0	9.0
Totals	60.0	22.3

Diet cost£ 1.72 per day Cost/litre 5.7 p

Diet intake is 135.6% of maximum predicted intake dry matter

Diet Composition (g/kg DM)

	Whole Diet	Concentrate Only
DM (g/kg)	371	
ME (MJ/kg DM)	10.1	13.0
Crude protein	167	210
Oil	40.4	
NDF	449.0	
Starch		14.9
Sugar		44.9
Long Roughage (%)	92.2	
DCAB (meq/kg DM)	523	
DUP (%MP requirement)	63.5	

Nutrient Balance (g/day)

	Animal requires	Diet supplies	Mineral Deficit
ME (MJ/day)	224	224	
ERDP	1688	2175	
DUP	859	1228	
RSV	113	251	

These feeding recommendations are produced as a guide only and do not constitute a guarantee that the predicted levels of performance will be achieved since many other factors have an effect on animal performance. In no event will SAC or the company using this program be liable for damages arising out of the use or misuse of this software.

Diet: Maize silage**Animal Details**

Weight:	600 kg animal
Dairy cow weight change:	0.00kg/day
Milk yield:	20 kg/day
Milk fat:	39 g/day
Milk protein:	34 kg/day
Weeks before calving:	40,
Weeks into lactation	12
Condition score:	2.5,
Lactation no.:	2

Recommended Diet

Feed	Fresh Weight (kg/d)	Dry Weight (kg/d)
Silage average	56.3	13.5
Maize silage	9.0	2.5
Totals	65.3	16.0

Diet cost£ 1.11 per day	Cost/litre	5.6 p
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Diet intake is 112.3% of maximum predicted intake dry matter

Diet Composition (g/kg DM)

	Whole Diet	Concentrate Only
DM (g/kg)	246	
ME (MJ/kg DM)	10.7	0
Crude protein	141	0
Oil	44.9	
NDF	463	
Starch	39.3	
Sugar	17.6	
Long Roughage (%)	100	
DCAB (meq/kg DM)	568	
DUP (%MP requirement)	42.6	

Nutrient Balance (g/day)

	Animal requires	Diet supplies	Mineral Deficit
ME (MJ/day)	170	171	
ERDP	1411	1524	
DUP	514	602	
RSV	103	262	

These feeding recommendations are produced as a guide only and do not constitute a guarantee that the predicted levels of performance will be achieved since many other factors have an effect on animal performance. In no event will SAC or the company using this program be liable for damages arising out of the use or misuse of this software.

Diet: LC med silage control**Animal Details**

Weight:	600 kg animal
Dairy cow weight change:	0.00kg/day
Milk yield:	25 kg/day
Milk fat:	39 g/day
Milk protein:	34 kg/day
Weeks before calving:	40,
Weeks into lactation	12
Condition score:	2.5
Lactation no.:	2

Recommended Diet

Feed	Fresh Weight (kg/d)	Dry Weight (kg/d)
Silage average	68.8	16.5
Maize silage	7.0	2.0
Totals	75.8	18.5

Diet cost£ 1.24 per day	Cost/litre	5.0 p
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Diet intake is 124.9% of maximum predicted intake dry matter

Diet Composition (g/kg DM)

	Whole Diet	Concentrate Only
DM (g/kg)	244	
ME (MJ/kg DM)	10.6	0
Crude protein	144	0
Oil	44.6	
NDF	462	
Starch	26.5	
Sugar	18.4	
Long Roughage (%)	100	
DCAB (meq/kg DM)	590	
DUP (%MP requirement)	44.9	

These feeding recommendations are produced as a guide only and do not constitute a guarantee that the predicted levels of performance will be achieved since many other factors have an effect on animal performance. In no event will SAC or the company using this program be liable for damages arising out of the use or misuse of this software.

Diet: 13/03/2012

Animal Details

Weight:	600 kg animal
Dairy cow weight change:	0.00kg/day
Milk yield:	30 kg/day
Milk fat:	39 g/day
Milk protein:	34 kg/day
Weeks before calving:	40,
Weeks into lactation	12
Condition score:	2.5,
Lactation no.:	2

Recommended Diet

Feed:	Fresh Weight (kg/d)	Dry Weight (kg/d)
Silage average	81.3	19.5
Maize silage	5.5	1.5
Totals	86.8	21.0
Diet cost£ 1.38 per day	Cost/litre 4.6 p	

Diet intake is 136.8% of maximum predicted intake dry matter

Diet Composition (g/kg DM)

	Whole Diet	Concentrate Only
DM (g/kg)	243	
ME (MJ/kg DM)	10.6	0
Crude protein	146	0
Oil	44.4	
NDF	461	
Starch	18.3	
Sugar	18.9	
Long Roughage (%)	100	
DCAB (meq/kg DM)	605	
DUP (%MP requirement)	47.4	

Nutrient Balance (g/day)

	Animal requires	Diet supplies	Mineral Deficit
ME (MJ/day)	224	224	
ERDP	1822	1997	
DUP	730	897	
RSV	113	262	

These feeding recommendations are produced as a guide only and do not constitute a guarantee that the predicted levels of performance will be achieved since many other factors have an effect on animal performance. In no event will SAC or the company using this program be liable for damages arising out of the use or misuse of this software.

Appendix 3 Conversion of LMD ppm-m to MJ/ day

$M_{TV} = M_{MD} \times TV_r$, where M_{TV} is ruminating breath enteric methane in ml, M_{MD} is the measured LMD enteric methane in ml, TV_r is the tidal volume during different animal activities.

$M_{TTA} = M_{TV} \times R_{TA}$, M_{TTA} is the amount of enteric methane emission at specific animal activity (ruminating, feeding, drinking etc.), R_{TA} is the time of activity.

$MD = T_D \times 1/R_{TA}$, M_D is daily enteric methane emission, T_D = time in seconds, using specific density conversion, and enteric methane emission is calculated as follows:

$M_{DG} = 0.000576 \times M_{TV} \times TV_r$, (Chagunda *et al.*, 2003), Tidal volume (lying) = 3100 ml, Tidal volume standing = 3800 ml (Tenney, 1982).

Calculation of mean square prediction error (MSPE), root mean square prediction error (RMSPE) and regression standard error (RSE)

$MSPE = [O-P]^2 / n$, $RMSPE = [(O-P)^2 / n]^{-1/2}$ (Ellis *et al.*, 2007),

Regression standard error = $[(O-P)^2 / n]^{-1/2}$ where O = observed value, P = Predicted value, n = number of values observed and predicted (Wilkerson *et al.*, 1995).